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TRANSMITTAL OF APPEAL BRIEF

Docket No.: CFBF-P02-002

In re Application of: Wagner, Denisa

Application No.	Filing Date	Examiner	Group Art Unit
08/948,393	10/10/97	Phillip Gambel	1644

Invention: METHOD FOR TREATING OR INHIBITING ATHEROSCLEROSIS WITH PSGL-1

TO THE COMMISSIONER OF PATENTS:

Transmitted herewith is the Reply Brief in this application, with respect to the Office Action mailed from the PTO on May 3, 2005 and the Notice of Appeal filed on November 3, 2005.

The fee for filing this Appeal Brief is $500.00 .

[x] Large Entity [ ] Small Entity

[ ] A petition for extension of time is also enclosed.

The fee for the extension of time is _____ .

[ ] A check in the amount of _____ is enclosed.

[x] Charge the amount of the fee (if any) to Deposit 50-3685 .
This sheet is submitted in duplicate.

[ ] Payment by credit card. Form PTO-2038 is attached.

[x] The Director is hereby authorized to charge any additional fees that may be required to Deposit Account No. 50-3685 .
This sheet is submitted in duplicate.

William Gosz

Dated: January 3, 2006

William G. Gosz
Attorney Reg. No. : 27,787
Gosz and Partners, LLP
450 Bedford Street
Lexington, MA 02420
(781) 863-1116

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: MS Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: 1/3/06 Signature: _____ (Patricia McKenney)

AP/ ZIW

Attorney Docket No. CFBF-P02-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant(s):	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	October 10, 1997		
For:	METHOD FOR TREATING OR INHIBITING ATHEROSCLEROSIS WITH PSGL-1		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Mail Stop Appeals, on 1/3/06.

Patricia McKenney

Mail Stop Appeal
Commissioner for Patents
P.O. 1450
Alexandria, VA. 22313-1450
ATTENTION: Board of Patent Appeals and Interferences

Sir:

APPELLANT'S BRIEF ON APPEAL

This is an appeal to the Board of Patent Appeals and Interferences (the "Board") from the decision of the Examiner finally rejecting claims 71, 81, 85, 87-89, 92 and 94-97, and is in furtherance of the Notice of Appeal filed on November 3, 2005, in this application. The appealed claims are as set forth in the attached Claims Appendix. Provision for the payment of fees required for filing this brief, and any required extension of time for filing the brief, are submitted herewith. This brief is submitted in triplicate in accordance with the provisions of 37 C.F.R. §1.192(a).

01/10/2006 TBESHAH1 00000029 503685 08948393

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REAL PARTY IN INTEREST

The real party in interest in this appeal is the CBR Institute for Biomedical Research, Inc., aka The Center for Blood Research, Inc., the assignee of the rights of the inventors in the above-identified patent application. The CBR Institute for Biomedical Research, Inc., is an affiliate of the Harvard Medical School.

RELATED APPEALS AND INTERFERENCES

There are believed to be no related appeals or interferences that will directly affect, be directly affected by, or have a bearing on the Board's decision in this appeal.

STATUS OF CLAIMS

The status of the claims in this application is as follows. Claims 71, 81, 85, 87-89, 92 and 94-97 are pending and are on appeal. Claims 1-70, 72-80, 82-84, 86, 90, 91 and 93 have been canceled. The limitations in claims 71 and 95 directed to mimetics of PSGL-1 has been withdrawn from consideration. Claims 71 and 95 are independent claims. Claim 71 is directed to methods for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on the arterial walls of a mammal. Claim 95 is directed to a method for treating atherosclerosis in a mammal which has been subjected to a vessel corrective technique.

STATUS OF AMENDMENTS

Claims 71, 81, 85, 87-89, 92 and 94-97 were finally rejected in the final Office Action of May 3, 2005. A Notice of Appeal was filed on November 3, 2005. No amendments have been made or entered following issuance of the final Office Action.

SUMMARY OF CLAIMED SUBJECT MATTER

Atherosclerosis is a principal cause of heart attacks among adults in the United States. This condition results from the restricted flow of arterial blood due to the accumulation of fibrous plaque over time in the arterial lumen. Death or incapacity of the subject may result from the rupture of the fibrous cap of the plaque, causing hemorrhage, thrombosis and occlusion of the artery. The fibrous plaque is formed from fatty streaks which develop into lesions composed predominantly of layers of smooth muscle cells, lipid-filled macrophages, and T cells. The earliest stages of atherosclerosis occur when migrating monocytes and T lymphocytes bind to the lumen of the arterial wall. Atherosclerosis is a chronic, long term condition, and is distinguished from more acute conditions such as local inflammation. Page 1, line 17 to page 2, line 22. Appellants have found that P-selectin is implicated in the origins of atherosclerosis as a result of the mediation of platelet or endothelial cell binding and adhesion to monocytes.

In one embodiment of the invention, as described in claim 71, the invention is directed to a method for treating or inhibiting atherosclerosis in a mammal by decreasing the formation or growth of plaque on arterial walls in a mammal. This is accomplished by administering to the mammal an agent selected from the group consisting of PSGL-1, soluble forms of PSGL-1, and fragments of PSGL-1. The agent is administered to the mammal in repeated sequential doses or by controlled release methods over a period of months or years, and is effective to inhibit the interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin. Page 3, lines 1-16; page 4, lines 23-28; page 5, lines 6-14 and 27-32; page 7, lines 1-3; and page 12, line 21 to page 14, line 2.

In another embodiment, as described in claim 95, the invention is directed to a method for treating atherosclerosis in a mammal by performing a vessel corrective technique on a mammal selected form the group consisting of angioplasty, stenting, atherectomy and bypass surgery on the mammal, and subsequently administering to the mammal an effective amount of an agent selected form the group consisting of PSGL-1, soluble forms of PSGL-1, and PSGL-1 fragments. The administration of the agent is accomplished over a period of months or years, and results in a decrease in the formation or growth of plaque on the arterial walls. Page 13, line 21 to page 14, line 2; and page 14, lines 20-29.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether claims 71, 81, 85, 87-89, 92 and 94-97 are unpatentable under 35 U.S.C. 103(a) as obvious over Cummings et al. (U.S. Patent No. 5,464,778) in view of Larsen et al. (U.S. Patent No. 5,840,679), Tedder et al. (U.S. Patent No. 5,834,425), Coller et al. (U.S. Patent No. 5,976,532), Sluiter et al. (*J. Cardiovascular Pharmacology*, 22 (Suppl. 4): S37-S44 (1993)), Aberg et al. (U.S. patent No. 5,061,694), Casscells et al. (U.S. Patent No. 5,308,622), Hinstridge et al. (*Drugs*, 42 (Suppl. 2): 8-2 (1991)), *The Merck Manual of Diagnosis and Therapy*, 16th Ed., pages 409-413 (1992), and De Felice et al. (*Angiology* 41:1-11 (1990))

2. Whether claims 71, 81, 85, 87-89, 92 and 94-97 are unpatentable based on the judicially created doctrine of obviousness-type double patenting in view of claims 40-41, 49-52, 59-60 and 73 of U.S. Patent Application No. 09/436,076.

ARGUMENTS

I. Rejection of Claims 71, 81, 85, 87-89, 92 and 94-97 as obvious over Cummings et al. in view of Larsen et al., Tedder et al., Coller et al., Sluiter et al., Aberg et al., Casscells et al., Hinstridge et al., *The Merck Manual of Diagnosis and Therapy*, and De Felice et al.

The primary reference cited by the Examiner, Cummings et al., discusses atherosclerosis in a section labeled "Clinical Applications". See col. 18, line 33 of the patent. In particular, the Cummings, et al. patent states that atherosclerosis is an example of a pathological condition in which an inflammatory response may occur, and that the P-selectin glycoprotein ligand can be used to treat such an inflammatory responses. See col. 18, lines 34-53 of the reference.

It is appellants' position that Cummings et al. is directed to the treatment of acute inflammatory conditions, such as ischemica and reperfusion, rather than atherosclerosis. The focal point of the reference is the prevention of leukocyte adherence to vascular endothelium. With regard to atherosclerosis, the reference makes the following comments, at col. 19, line 64 to col. 20, line 5:

> "Platelet-leukocyte interactions are believed to be important in atherosclerosis. Platelets might have a role in recruitment of monocytes into atherosclerotic plaques; the

accumulation of monocytes is known to be one of the earliest detectable events during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia."

These comments do not teach or suggest that the reference contemplates the use of PSGL-1 for reducing the formation of arterial plaque. Rather, the reference is directing one skilled in the art to the treatment of thrombus (blood clot) formation. Such treatment would involve the prevention of platelet activation by leukocytes as described elsewhere in the reference. A reduction is plaque formation is not inherent in the treatment of a thrombosis since plaque reduction would require a treatment regime of months or years.

Furthermore, Cummings et al. is directed to the prevention of platelet activation in the circulatory system, rather that the inhibition of endothelial cell binding which is an essential component of atherosclerosis. See, in particular, the Wagner (II)132 Declaration, at paragraphs 4, 5 and 6. Thus, it is appellants' position that one skilled in the art, reading the Cummings et al. reference, would have no reasonable expectation that PSGL-1 could be used to reduce plaque formation, and further, that a long term treatment regime would be required to achieve this result.

Appellants also respectfully submit that the Cummings, et al. reference has been antedated as a result of the prior conception and subsequent reduction to practice of the claimed invention, coupled with the requisite diligence, as shown in the Wagner 131 Declaration.

Larsen et al., like Cummings et al., does not relate to the treatment of chronic conditions, such as atherosclerosis, but is instead directed to the treatment of inflammatory or acute conditions. Contrary to the position taken in the Office Action, neither Cummings et al. nor Larsen et al. disclose that a treatment for atherosclerosis can be administered in conjunction with a vessel-corrective technique.

Coller et al. relates to the treatment of a thrombotic condition using antibodies to GPIIb/IIIa. The present invention, in contrast, relates to the use of PSGL-1, and variants therefore, rather than antibodies. Cummings et al. does not teach the use of vessel corrective techniques, and does not teach the use of antibodies for therapeutic purposes. Consequently, applicants maintain that there is no basis for combining the Coller et al. and Cummings et al. references.

The Sluiter et al. reference has been cited to provide further evidence that one skilled in the art would have targeted the inhibition of P-selectin-mediated events for inhibiting leukocyte adhesion receptors to alleviate tissue damage in cardiovascular diseases. However, although the Sluiter et al. reference mentions P-selectin in a general sense, there is no disclosure in the reference concerning the inhibition of P-selectin binding to the ligand of P-selectin. In fact, the Sluiter et al. reference is actually directed to the possible role of oxygen-derived free radicals in the treatment of inflammation. See the Summary portion of the reference on page S37, and the discussion on page S38. Accordingly, the Sluiter et al. reference does not oversome the shortcomings of Cummings et al.

The remaining references cited by the Examiner do not cure the deficiencies of the Cummings et al. Larsen et al., Coller et al. and Sluiter et al. references as discussed above. references. In particular, the Aberg et al., Casscells et al. and Hinstridge et al. references do not relate to the use of appellant's agent for the treatment of diseases. Accordingly, it would be entirely speculative to suggest that the use of appellants' particular agents for the treatment of atherosclerosis can be administered over a prolonged period of time, and that such treatment would have beneficial results.

Similarly, the Merck and De Felice et al. references are apparently relied upon for linking atherosclerosis with a decrease in plaque growth or formation. Of course, appellants do not claim to have discovered the scientific basis for atherosclerosis. Rather, appellants have developed a treatment protocol for atherosclerosis which is not disclosed or suggested in any of the cited references.

Finally, appellants note that the sheer number of references required for formulating the present obviousness rejection (a total of 10 references) is itself a strong indication that the present claims are not obvious.

II. Rejection of Claims 71, 81, 85, 87-89, 92 and 94-97 as unpatentable based on obviousness-type double patenting in view of claims 40-41, 49-52, 59-60 and 73 of U.S. Patent Application No. 09/436,076).

U.S. Patent Application No. 09/436,076, which forms the basis of this rejection, has now been abandoned. Accordingly, this rejection is now moot.

Summarizing, for the reasons presented in this brief, appellants respectfully urge the Board to reverse the rejection made in the Final Office Action, and to allow all of the appended claims.

Appellants hereby authorize the Commissioner, to debit the $500.00 fee for filing this appeal brief from Appellant's Deposit Account No. 18-1945. If there are any other fees not accounted for above, Appellants hereby authorize the Commissioner to charge the fee to Deposit Account 18-1945.

Respectfully submitted,
GOSZ AND PARTNERS

Date: 01/03/05

William Gosz

William G. Gosz
Reg. No. 27,787
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450 Bedford Street
Lexington, MA 02420

CLAIMS APPENDIX

71. A method for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on arterial walls in a mammal comprising:

providing an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, said P-selectin being on an endothelial cell; and

administering said agent to a mammal in need of such treatment so as to cause such inhibition to occur, said agent being administered repeatedly in sequential doses or by the controlled release to the mammal over a period of months or years,

wherein said agent is selected from the group consisting of PSGL-1, soluble forms of PSGL-1, fragments of PSGL-1, and mimetics of PSGL-1 which resemble PSGL-1 in shape and charge distribution, said agent being effective to inhibit the interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin.

81. The method of claim 71 wherein said P-selectin can bind to said PSGL-1 in the absence of said agent.

85. The method of claim 71 wherein said agent is administered in combination with other therapeutic agents.

87. The method of claim 71 wherein said mammal is human.

88. The method of claim 71 wherein said agent is administered in a dose of from about 0.01 mg/kg to about 200mg/kg of body weight.

89. The method of claim 71 wherein said agent is administered at a dose of about 100 mg/kg of body weight.

92. The method of claim 95, wherein said vessel-corrective technique is selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery.

94. The method of claim 95, wherein said agent is administered in combination with other therapeutic agents.

95. A method for treating atherosclerosis in a mammal to which a vessel-corrective technique is administered comprising:

performing a vessel-corrective technique selected form the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery on a mammal; and

administering to said mammal, after said vessel-corrective technique, an effective amount of an agent selected from the group consisting of PSGL-1, soluble forms of PSGL-1, fragments of PSGL-1, and mimetics of PSGL-1 which resemble PSGL-1 in shape and charge distribution, said agent being administered repeatedly in sequential doses or by the controlled release to the mammal over a period of months or years to decrease the formation or growth of plaque on the arterial walls of the mammal.

96. The method of claim 71 wherein the agent is administered over a period of years.

97. The method of claim 95 wherein the agent is administered over a period of years.

EVIDENCE APPENDIX

1. Declaration of Denisa Wagner and Robert Johnson Under Rule 131*

2. Declaration of Denisa Wagner under Rule 132**

* Considered and entered by the Examiner on March 18, 2003.

** Considered and entered by the Examiner on February 25, 2005.

Ex. No. 1

ATTORNEY DOCKET NO. CFBF-P03-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wagner et al.
Examiner: P. Gambel
Serial No.: 08/436,076
Art Unit: 1644
Filing Date: November 8, 1999
For: METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Washington, D.C. 20231 on 3/12/03.

Patricia McKenney

BOX AMENDMENT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231
Dear Sir:

DECLARATION UNDER 37 CFR 1.131

We, Denisa D. Wagner and Robert C. Johnson, declare and state as follows:

1. We are the applicants of the above-identified patent application, and the co-inventors of the subject matter disclosed and claimed therein.

2. We are familiar with the present claims of the above-identified application, which are directed to methods for treating or inhibiting atherosclerosis in a mammal by administering an agent that inhibits an interaction between P-selectin and PSGL-1 and E-selectin and a ligand of E-selectin., e.g. PSGL-1 (P-selectin glycoprotein ligand-1), soluble forms of PSGL-1, fragments of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

3. We conceived the claimed invention at least as early as 1988, and coupled with due diligence from a time prior to November 16, 1992, reduced the claimed in invention to practice at least as early as May 6, 1994.

4. Exhibit A is a copy of a page showing a note authored by co-inventor Denisa Wagner in 1988. The notes shown in the Exhibit were recorded by Dr. Wagner during the conference of the American Heart Association held in 1988, and were written on the last page of the program booklet next to a listing of meetings to be held in 1989. The note on the bottom right hand side of the page states that

> Macrophages (Mφ) eat bits of activated platelets. ELAM-1 = Padgem. Do monocytes bind to Padgem on platelets. Padgem is an opsomizing agent to get rid of debris of platelets.

The term "Padgem" here refers to P-selectin and the term "ELAM-1" refers to E-selectin (Endothelial Leukocyte Adhesion Molecule). In 1988, E-selectin was known to mediate endothelial binding to leukocytes. We conceived that there is a functional relationship between E-selectin and P-selectin, and that P-selectin mediates the binding of platelets to macrophages (leukocytes implicated in atherosclerosis).By binding to Padgem, the macrophages are "eating" bits of activated platelets, thereby increasing the fat (lipid) content of the macrophages, and promoting their conversion into foam cells (macrophage cells with a "foamy" appearance due to the presence of lipids that act as precursors for atherosclerotic plaque). Exhibit A thus demonstrates that we had identified a role for P-selectin and E-selectin in some of the key pathological events involved in atherosclerosis, e.g. macrophage binding to P-selectin on platelets, from a time well before November 16, 1992.

5. Exhibit B, also written by Dr. Wagner, describes an experiment we conceived on February 28, 1992. Exhibit B states:

> Breed P-selectin deficient mouse with a mouse strain that develops atherosclerosis. See if it (atherosclerosis) can be prevented.

According to this proposed experiment, a mouse deficient in P-selectin would be bred with a mouse strain that develops atherosclerosis to determine whether atherosclerosis can be prevented. In other words, we conceived that if P-selectin/ligand binding and/or E-selectin/ligand binding could be inhibited *in vivo* in a mammal, the atherosclerotic lesions could be reduced or inhibited. In order to complete this experiment, we understood that it would first be necessary to prepare a P-selectin knock-out mouse, and breed this mouse with mouse strains susceptible to atherosclerosis. It is known that mice are generally resistant to developing atherosclerosis. The mouse strain most susceptible to developing atherosclerosis is the C57 black mouse. But the C57 mouse must still be fed a high lipid diet to observe any meaningful development of atherosclerosis.

6. Exhibit C, also written by Dr. Wagner, describes a proposal we conceived on March 2, 1992, to study the role of the P-selectin in atherosclerosis by developing a suitable mouse model, and feeding the P-selectin deficient mice (mutants) and control wild-type mice (P-selectin positive) with a lipid diet. The, formation of atherosclerotic lesions in the mice would be studied and characterized. Exhibit C states, on page 5:

> Study the role of P-selectin in atherosclerosis by feeding P-selectin deficient and P-selectin positive mice a lipid diet. Study the formation of atherosclerotic lesions in mice.

Page 5 of Exhibit C also poses the question whether von Willebrand (vW) disease pigs may be resistant to atherosclerosis because of a lack of P-selectin. P-selectin is stored in granules containing vW factor, and these granules are absent in vW disease.

7. At a time prior to November 16, 1992, we undertook to prepare a mouse model for subsequent testing. The mouse model took at least 4 years to prepare, and was completed on or about September 13, 1993. In order to prepare the mouse model, we used a knock-out mouse deficient in P-selectin and back-crossed this mouse with C57 black mice. In order to be sure that the resulting mutant mouse would be susceptible to atherosclerotic lesion development, we

decided to breed 4 generations of mice, with each generation being more susceptible to atherosclerosis. First we developed a P-selectin deficient mouse. Then we bred the P-selectin deficient mouse with a C57 black mouse. Finally, we bred the offspring of the first breeding with another C57 black mouse, and so on for a total of 4 back-cross breedings. We reasoned that the fourth generation would be suitable for evaluation. It took us about 3 years to make a P-selectin deficient mouse, and another year to complete the back-crossing process with the C57 black mice. This work was laborious and continuous, and consumed a large amount of our time and effort. Although the general technology for creating mouse models had been developed by others, we were the first to develop a P-selectin-deficient mouse model. We diligently worked on successfully constructing such a model, and verifying the correct properties and characteristics of the mutant mouse by about September 13, 1993.

8. After the preparation of the mutant mouse deficient in P-selectin on the C57 black background, we promptly commenced feeding the mice (control and experimental) a diet high in lipids. The experimental and control mice were fed a lipid diet for approximately eight months prior to sacrificing the animals and recording the data, This took approximately 8 months since even the C57 black mice are somewhat resistant to the formation of atherosclerosis. Immediately thereafter, we sacrificed the animals and evaluated them for the size and character of atherosclerotic lesions. We prepared the table enclosed as Exhibit D on May 6, 1994. The table in Exhibit D shows the size of atherosclerotic lesions in P-deficient (mutant) mice compared to wild type mice as controls. These results demonstrate a reduction in the size of atherosclerotic lesions in P-selectin deficient mice. Based on these results we concluded that inhibitors of P-selectin/ligand binding and/or E-selectin/ligand binding would be useful for the treatment or inhibition of atherosclerosis, and this constitutes an actual reduction to practice of the claimed invention.

9. From the above information, we deduced that inhibitors of P-selectin and/or E-selectin could be used to treat atherosclerosis in mammals based on the role of P-selectin and/or E-selectin on the pathogenesis of atherosclerosis as presently claimed in the above-identified application. We further believe that the above information constitutes evidence the claimed

invention was conceived prior to November 16, 1992, and diligently reduced to practice at least as early as the actual reduction to practice date of May 6, 1994.

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/25/2003
Date

Denisa D. Wagner

3-6-03
Date

Robert C. Johnson

EXHIBIT A
Page 1 of 1

AMERICAN HEART ASSOCIATION CME OFFERINGS
1989 Highlights

For information contact the American Heart Association, Scientific Sessions, 7320 Greenville Avenue, Dallas, Texas 75231.

***SCIENTIFIC CONFERENCE ON MEMBRANE EVENTS AND INTRACELLULAR SIGNALLING IN THE CARDIOVASCULAR SYSTEM**
Waikoloa, Hawaii
AHA Council on Basic Science and the Japanese Heart Foundation
January 7-11, 1989
Conference Chairman: James T. Stull, PhD

14TH INTERNATIONAL JOINT CONFERENCE ON STROKE AND CEREBRAL CIRCULATION
San Antonio, TX
AHA Council on Stroke
February 9-11, 1989
Conference Chairman: Vladimir C. Hachinski, MD

SCIENTIFIC CONFERENCE ON CORONARY ATHEROSCLEROSIS AND THROMBOSIS
Keystone, CO
AHA Councils on Circulation, Atherosclerosis, Thrombosis, and Clinical Cardiology
February 22-25, 1989
Conference Chairman: Paul J. Cannon, MD

2ND INTERNATIONAL CONFERENCE ON PREVENTIVE CARDIOLOGY AND THE ANNUAL MEETING OF THE AHA COUNCIL ON EPIDEMIOLOGY
Washington, DC
AHA Council on Epidemiology
June 18-22, 1989
Conference Chairman: Jeremiah Stamler, MD

***15TH TEN-DAY SEMINAR ON THE EPIDEMIOLOGY AND PREVENTION OF CARDIOVASCULAR DISEASES**
Tahoe City, CA
AHA Council on Epidemiology
July 30-August 12, 1989
Conference Chairman: Darwin R. Labarthe, MD, PhD

43RD ANNUAL FALL CONFERENCE AND SCIENTIFIC SESSIONS OF THE COUNCIL FOR HIGH BLOOD PRESSURE RESEARCH
Cleveland, OH
AHA Council for High Blood Pressure Research
September 26-29, 1989
Conference Chairman: Allen W. Cowley, Jr, PhD

62ND SCIENTIFIC SESSIONS
New Orleans, LA
AHA Scientific Councils
November 13-16, 1989
Conference Chairman: Michael R. Rosen, MD

*Limited attendance

do plt release by ↓ blocks w 125 β inhibit B/β 15-42

put flow through of ϕ column to on to fibrin column. ϕ rec. should be reduced. control do it reverse go back to fibrin clots does after reaching w fibrin the receptor get phosphorylated

Put on column EC grown in *Phosphate I stimulation w fibrin, EDTA elute If this works to A23 release ϕ incubation etc. Do x-linking - it will work

Add ϕ to all lysate put on fibrin column. ϕ should inhibit $IIb\,IIIa$-like binding but fibrin specific b. should not be affected !!

elute w RGD, b. to fibrin may not be through RGD or elute w γ pept.

see if severe vWd plts plts have Padgem

m ϕ eat bits of activated plts ELAM-1 ! = Padgem

do monocytes b. to Padgem on plts Padgem is an opsonizing agent to get rid of depress ct plts

A23187 makes these vesicles. are these some of Padgem ?

EXHIBIT B
Page 1 of 1

feb. 28/92

Bread OP-ed mouse with a mouse strain that develops atherosclerosis see if it can be prevented.

projects for Bob

3/2/92

Prepare antibodies to P-s. cytoplasmic tail (polyclonal). Do they recognise other granular proteins → clone them

Schaffhausen: Abs to SH2 domain of PDGF rec. binds to other prot. containing this homologous domain

Role of dibasic cleavage site in targeting to storage granules

Insulin c-DNA is available that has both sites mutated. When expressed in AtT-20 cells will it be stored?

Randy Kaufman has a protein inhibitor of PACE (and likely related enzymes. It could be transfected into cells and see if storage is prevented (ACTH, vWf etc).

In endothelial cells that do not express vWf – what happens to P-sel

a) culture EC in the presence

Role of vicinal cysteines in integrins matrix assembly?

Targeting of P-selectin in yeast

Is there a storage compartment in yeast

use yeast secretion mutants and clathrin ⊖ cells to find the cellular machinery responsible for targeting of transmembrane proteins.

In the absence of vWf in EC, what happens to P-sel?

a) use vWdisease pigs EC
does our Ab x-react?
disadvantage: availability
advantage - normal platelets: vWd pts

b) use #UVEC grown in the presence of antisense oligos to vWf
→ inhibit vWf synthesis
see where P-sel is and if it can be transported to cell surface
+/- secretagogues

vWdisease pigs are resistant to atherosclerosis. Is this an effect of vWf (lack of) or P-selectin absence?

Study role of P-S. in atherosclerosis by feeding ⊖ P-S and ⊕ P-S lipid diet to mice → formation of atherosclerotic lesions

5/6/94 Denisa Bob

	Genotype	Score
271	Mut	−
278	WT	++ 2.7
279	Mut	−
137	Mut	−
119	WT	+ 0.5
548 E	Mut	+ 0.2
269	WT	++
270	Mut	+ small
40	WT	+++
34	Mut	−
35	WT	+
19	WT	+ (Maybe 2+)
20	WT	2+
268	WT	+++
42	Mut	−
106	WT	3+++
18	Mut	−
89	WT	−

Rachelle A. Rosenbaum 5/9/94
City of Boston
Suffolk County
a true copy of the original

MY COMMISSION EXPIRES JUNE 6, 1997

** See when it is deep lesion + not raised it is not a lesion. #34

#19 Must be positive about lesion.
Not good

EXHIBIT D
Page 1 of 1

Ex. No. 2

Attorney Docket No. CFBF-P02-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant(s):	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	November 8, 1999		
For:	METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS WITH PSGL-1		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, P.O. 1450, Alexandria, VA. 22313-1450 on February 22, 2005.

Patricia McKenney

Commissioner for Patents
P.O. 1450
Alexandria, VA. 22313-1450

DECLARATION UNDER RULE 132

Sir:

I, Denisa D. Wagner, declare and state as follows:

1. I am a Professor in the Department of Pathology at Harvard Medical School, and a Senior Investigator at The CBR Institute for Biomedical Research, Inc., Boston, Massachusetts. My Curriculum Vitae is attached hereto as an Exhibit. I am also an inventor of the above-identified patent application. I consider myself to be an expert in the field of cardiovascular medicine and pathology, as reflected in my Curriculum Vitae, and I am well aware of the knowledge level of others skilled in this art.

2. I have reviewed the outstanding Office Action of October 21, 2004, in the above-identified patent application. I am also familiar with the claims of this application, as presently amended, which are directed to methods for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on arterial walls. This is accomplished by administering PSGL-1, or selected variants thereof, to a subject over a prolonged period of time, i.e. months or years.

3. I am familiar with the references cited by the Examiner in the outstanding Office Action. In particular, I have reviewed the Cummings et al. reference (U.S. Patent No. 5,464,778), which I understand to be the primary reference cited in the Office Action.

4. The Cummings et al. reference is generally directed to inflammatory thrombotic conditions such as ischemia and reperfusion. In col. 19, line 64 to col. 20, line 5, the Cummings et al. reference makes the following disclosure relating to atherosclerosis and platelet-leukocyte interactions:

> "Platelet-leukocyte interactions are believed to be important in atherosclerosis. Platelets might have a role in recruitment of monocytes into atherosclerotic plaques; the accumulation of monocytes is known to be one of the earliest detectable events during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia."

5. My interpretation of the above cited passage is as follows. Cummings et al. speculate that platelet-leukocyte interactions are important in atherosclerosis. In fact, it is now well established that the key in atherosclerotic lesion development is the direct binding of monocytes to endothelial cells, and the reference does not discuss this. Cummings et al. discusses events following plaque rupture. Such events include thrombus formation leading to ischemic injury causing neutrophil recruitment. This event occurs long after plaque formation which is subject of the present application. The claims of our application specify that the P-selectin is on endothelial cells. Endothelial cells coat the arterial wall, and are not part of the

circulatory system as are the platelets. The plaque rupture, thrombotic events and neutrophil recruitment to the ischemic area discussed in the reference are not part of the present application.

6. I believe that the present invention can be distinguished from the Cummings et al. reference in the following respects. The present invention is directed to the treatment of atherosclerosis by decreasing the formation or growth of plaque on arterial walls. Atherosclerosis is a chronic condition caused by many factors, primarily by excessive plasma cholesterol levels, and results in the deposition of lesions and plaque on arterial walls. The treatment of atherosclerosis requires the long term administration of a medication to a subject in order to result in a meaningful improvement of the condition. This contrast with the treatment of a thrombosis, as disclosed in the Cummings et al. reference, which requires the commencement of an immediate treatment regime in order to prevent the reoccurrence of a thrombotic attack.

7. I also believe that the ability to design a mimetic of PSGL-1 having similar inhibitory characteristics, i.e. the ability to inhibit P-selectin, would be within the skill of a person in the art. Such a mimetic would optimally be designed based on a similarity of charge and shape as stated in the present claims.

8. Based on my knowledge, training and experience, it is my opinion that the references cited in the outstanding Office Action would not teach or suggest the method for treating atherosclerosis as stated in the present claims of the above-identified patent application.

I further declare that statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 2/18/05

Denisa D. Wagner, Ph.D.

CURRICULUM VITAE

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PLACE OF BIRTH: Prague, Czechoslovakia; U.S. citizen

EDUCATION: Universite de Geneve, Switzerland - Biochemistry
Diploma of Biochemistry, 1975, with distinction

Massachusetts Institute of Technology, Cambridge, MA
Biology - Ph.D., 1980

Harvard University, Cambridge, MA
M.A. (honorary), 1997

FACULTY POSITIONS:

Professor of Pathology, Harvard Medical School, Boston, MA.
1997-present.

Senior Investigator, The CBR Institute for Biomedical Research (formerly known as The Center for Blood Research), Boston, MA.
1994-present.

Associate Professor of Pathology, Harvard Medical School, Boston, MA.
1994-1997.

Associate Professor of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA. 1989-1994.

Associate Professor of Medicine, Tufts University School of Medicine and Member, Special and Scientific Staff, New England Medical Center, Boston, MA. 1987-1994.

Assistant Professor of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1985-1987.

Assistant Professor of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1982-1987.

Senior Instructor in Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1981-1982.

AWARDS:

Established Investigator Award, American Heart Association, Biosynthesis of von Willebrand protein by endothelial cells. 1986-1991.

XIth ISTH Congress award in recognition of an outstanding communication, 1987.

Gwendolyn J. Stewart Memorial Award to honor women in the biomedical sciences, 1998.

Special recognition award from the Council on Arteriosclerosis, Thrombosis and Vascular Biology, AHA, 1998

MERIT award, National Heart, Lung and Blood Institute, NIH, 1998-2008.

Sol Sherry Lecture, American Heart Association, 2004.

MAJOR COMMITTEE ASSIGNMENTS:

University:

1991-1994	Member of the Graduate Advisory Committee of the Graduate Program in Cell, Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University
1992-1994	Sackler School Committee on Programs and Faculty, Tufts University
1992-1994	Graduate Admission Committee of the Graduate Program in Cell, Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University
1995-Present	Member of the Graduate Program in Biological and Biomedical Sciences, Harvard Medical School
1998-Present	Member, Committee for Immunology, Program in Immunology, Harvard Medical School
1999-2002	Member of the Faculty Fellowship Committee, Harvard Medical School
2001-2004	Member, Standing Committee on Promotions, Reappointments, and Appointments, Harvard Medical School
2003-Present	Elected Member, Harvard Medical School Faculty Council

National and Regional:

Served on many review committees and panels for the National Institutes of Heath, American Heart Association, Juvenile Diabetes Foundation and American Red Cross.

Currently permanent member, NIH, NHLBI Thrombosis and Hemostasis Study Section (2002-2006)

MEMBERSHIPS, OFFICES, AND COMMITTEE ASSIGNMENTS IN PROFESSIONAL SOCIETIES:

1980-Present	American Society for Cell Biology
1982-Present	American Society of Hematology
1982-Present	International Society of Thrombosis and Haemostasis
1983-1997	Council on Thrombosis, American Heart Association
1985-Present	International Society of Thrombosis and Haemostasis, subcommittee on von Willebrand factor
1991-1996	American Heart Association, Vascular Wall Biology Study Committee

1992-Present	American Heart Association, Council on Thrombosis Executive Committee
1993-1995	American Heart Association, Council on Thrombosis Long-Range Planning Committee
1994-1996	American Heart Association, Council on Thrombosis Membership Committee (Chairman)
1994-Present	American Association for the Advancement of Science
1994-Present	North American Vascular Biology Organization (Founding Member)
1995-1998	American Society of Hematology, Scientific Subcommittee on Thrombosis & Vascular Biology
1997-Present	North American Vascular Biology Organization (Councilor)
1997-Present	Council on Arteriosclerosis, Thrombosis and Vascular Biology, American Heart Association (Fellow)
1998	Council of the Gordon Research Conferences (Member)
1998-Present	The Molecular Medicine Society (Member)
1999-Present	Boston Obesity Nutrition Research Center (Member)
2001-Present	National Hemophilia Foundation (Member)
2001-2005	American Society of Hematology, Scientific Committee on Thrombosis & Vascular Biology (Member)
2002-Present	Harvard Center for Neurodegeneration & Repair (Member)
2004-2010	Council of the International Society on Thrombosis and Haemostasis (Member)
2004-2005	Chair, Scientific Committee on Thrombosis and Vascular Biology, American Society of Hematology

EDITORIAL BOARDS:

1993-2004	Molecular Biology of the Cell
1994-1999	Journal of Clinical Investigation
1998-Present	Molecular Medicine
2003-2008	Blood

PUBLICATIONS
DENISA D. WAGNER, Ph.D.

1. Hynes RO, Ali IU, Destree AT, Mautner V, Perkins ME, Senger DR, **Wagner DD** and Smith KK. A large glycoprotein lost from the surfaces of transformed cells. Ann NY Acad Sci 312:317-342, 1978.

2. **Wagner DD** and Hynes RO. Domain structure of fibronectin and its relation to function (disulfides and sulfhydryl groups). J Biol Chem 254:6746-6754, 1979.

3. Hynes RO, Destree AT, Perkins ME and **Wagner DD.** Cell surface fibronectin and oncogenic transformation. J Supramolecular Str 11:95-104, 1979.

4. **Wagner DD** and Hynes RO. Topological arrangement of the major structural features of fibronectin. J Biol Chem 255:4304-4312, 1980.

5. **Wagner DD,** Ivatt R, Destree AT and Hynes RO. Similarities and differences between fibronectins of normal and transformed hamster cells. J Biol Chem 256:11708-11715, 1981.

6. **Wagner DD** and Hynes RO. Fibronectin coated beads are endocytosed by cells and align with microfilament bundles. Exp Cell Res 140:373-381, 1982.

7. Van De Water L III, **Wagner DD,** Crenshaw EB III and Hynes RO. Fibronectin-dependent endocytosis by macrophage-like ($P388D_1$) and fibroblastic (NIL 8) cells. In: Cellular Recognition. Glaser L, Frazier W and Gottlieb D (Eds.). New York: Alan R. Liss, 869-878, 1982.

8. Hynes RO, Destree AT and **Wagner DD.** Relationships between microfilaments, cell-substratum adhesion and fibronectin. Cold Spring Harbor Symposia on Quantitative Biology 46:659-669, 1982.

9. **Wagner DD,** Olmsted JB and Marder VJ. Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. J Cell Biol 95:355-360, 1982.

10. **Wagner DD** and Marder VJ. Biosynthesis of von Willebrand protein by human endothelial cells: identification of a large precursor polypeptide chain. J Biol Chem 258:2065-2067, 1983.

11. **Wagner DD,** Urban-Pickering M and Marder VJ. von Willebrand protein binds to extracellular matrices independently of collagen. Proc Natl Acad Sci USA 81:471-475. 1984.

12. Sporn LA, Rubin P, Marder VJ and **Wagner DD.** Irradiation induces release of von Willebrand protein from endothelial cells in culture. Blood 64:567-570, 1984.

13. **Wagner DD** and Marder VJ. Biosynthesis of von Willebrand protein by human endothelial cells: processing steps and their intracellular localization. J Cell Biol 99:2123-2130, 1984.

14. Sporn LA, Chavin SI, Marder VJ and **Wagner DD.** Biosynthesis of von Willebrand protein by human megakaryocytes. J Clin Invest 76:1102-1106, 1985.

15. **Wagner DD,** Mayadas T, Urban-Pickering M, Lewis BH and Marder VJ. Inhibition of disulfide bonding of von Willebrand protein by monensin results in small, functionally defective multimers. J Cell Biol 101:112-120, 1985.

16. Fay PJ, Kawai Y, **Wagner DD,** Ginsberg D, Bonthron D, Ohlsson-Wilhelm BM, Chavin SI, Abraham GN, Handin RI, Orkin SH, Montgomery RR and Marder VJ. Propolypeptide of von Willebrand factor circulates in blood and is identical to von Willebrand antigen II. Science 232:995-998, 1986.

17. Sporn LA, Marder VJ and **Wagner DD.** Inducible secretion of large biologically potent von Willebrand factor multimers. Cell 46:185-190, 1986.

18. **Wagner DD,** Mayadas T and Marder VJ. Initial glycosylation and acidic pH in the Golgi apparatus are required for multimerization of von Willebrand factor. J Cell Biol 102:1320-1324, 1986.

19. **Wagner DD,** Lawrence SO, Ohlsson-Wilhelm BM, Fay PJ and Marder VJ. Topology and order of formation of interchain disulfide bonds in von Willebrand factor. Blood 69:27-32, 1987.

20. **Wagner DD,** Fay PJ, Sporn LA, Sinha S, Lawrence SO and Marder VJ. Divergent fates of von Willebrand factor and its propolypeptide (von Willebrand antigen II) after secretion from endothelial cells. Proc Natl Acad Sci USA 84:1955-1959, 1987.

21. Sporn LA, Marder VJ and **Wagner DD.** von Willebrand factor released from Weibel-Palade bodies binds more avidly to extracellular matrix than that secreted constitutively. Blood 69:1531-1534, 1987.

22. Sinha S and **Wagner DD.** Intact microtubules are necessary for complete processing, storage and regulated secretion of von Willebrand factor by endothelial cells. Eur J Cell Biol 43:377-383, 1987.

23. Ribes JA, Francis CW and **Wagner DD.** Fibrin induces release of von Willebrand factor from endothelial cells. J Clin Invest 79:117-123, 1987.

24. Mayadas TN, **Wagner DD** and Simpson PJ. von Willebrand factor biosynthesis and partitioning between constitutive and regulated pathways of secretion after thrombin stimulation. Blood 73:706-711, 1989.

25. Handin RI and **Wagner DD.** von Willebrand factor: molecular and cellular biology. In: Progress in Hemostasis and Thrombosis (vol. 9). Coller B (Ed.). Philadelphia: W B Saunders, 233-259, 1989.

26. Roarke MC, **Wagner DD,** Marder VJ and Sporn LA. Temperature-sensitive steps in the secretory pathway for von Willebrand factor in endothelial cells. Eur J Cell Biol 48:337-343, 1989.

27. Sporn LA, Marder VJ and **Wagner DD.** Differing polarity of the constitutive and regulated secretory pathways for von Willebrand factor in endothelial cells. J Cell Biol 108:1283-1289, 1989.

28. Bonfanti R, Furie BC, Furie B and **Wagner DD.** PADGEM (GMP-140) is a component of Weibel-Palade bodies of human endothelial cells. Blood 73:1109-1112, 1989.

29. Avissar N, Whitin JC, Allen PZ, **Wagner DD,** Liegey P and Cohen HJ. Plasma selenium dependent glutathione peroxidase: cell of origin and secretion. J Biol Chem 264:15850-15855, 1989.

30. Ribes JA, Ni F, **Wagner DD** and Francis CW. Mediation of fibrin-induced release of von Willebrand factor from cultured endothelial cells by the fibrin β chain. J Clin Invest 1989, 84:435-442, 1989.

31. Mayadas TN and **Wagner DD.** In vitro multimerization of von Willebrand factor is triggered by low pH: importance of the propolypeptide and free sulfhydryls. J Biol Chem 264:13497-13503, 1989.

32. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, **Wagner DD** and Furie B. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 59:305-312, 1989.

33. **Wagner DD.** Storage and secretion of von Willebrand factor. In: Role of Factor VIII and von Willebrand Factor. Zimmerman TS and Ruggeri ZM. (Eds.). New York: Marcel Dekker, pp. 161-180, 1989.

34. Larsen E, Palabrica T, Sajer S, Gilbert GE, **Wagner DD,** Furie BC and Furie B. PADGEM-dependent adhesion of activated platelets to monocytes and neutrophils is mediated by a lineage-specific carbohydrate, lacto-N-fucopentaose III (CD15). Cell 63:467-474, 1990.

35. **Wagner DD.** Cell biology of von Willebrand factor. In: Annual Review of Cell Biology. Palade GE, Alberts BM and Spudich JS. (Eds). 6:217-246, 1990.

36. Peters JH, Sporn LA, Ginsberg MH and **Wagner DD.** Human endothelial cells synthesize, process, and secrete fibronectin molecules bearing an alternatively spliced type III homology (ED1). Blood 75:1801-1808, 1990.

37. Mayadas TN and **Wagner DD.** von Willebrand factor biosynthesis and processing. Ann NY Acad Sci, 614:153-166, 1991.

38. Furie B, Celi A, Palabrica TM, Larsen E, **Wagner DD** and Furie, BC. PADGEM a Leukocyte Receptor on Activated Platelets: Biology and application to in vivo medical diagnostics. In: Biotechnology of Plasma Proteins: Haemostasis, Thrombosis and Iron Proteins. Albertini A, Lenfant CL, Mannucci PM, and Sixma JJ. (Eds). Basel: Karger, 32-36, 1991.

39. **Wagner DD** and Bonfanti R. von Willebrand factor and the endothelium. Mayo Clinic Proceedings 66:621-627, 1991.

40. Bevilacqua E, Butcher B, Furie B, Furie B, Gallatin M, Gimbrone M, Harlan J, Kishimoto K, Lasky L, McEver R, Paulson J, Rosen S, Seed B, Springer T, Stoolman L, Tedder T, Varki A, **Wagner DD,** Weissman I and Zimmerman G. Letter to the Editor. Selectins: A family of adhesion receptors. Cell 67:233, 1991.

41. **Wagner DD.** Structure and biology of von Willebrand factor. In: Hematology: Basic Principles and Practices. Benz EJ, Cohen HJ, Furie B, Hoffman R and Shattil SJ. (Eds.). New York: Churchill Livingstone, 1354-1358, 1991.

42. **Wagner DD,** Saffaripour S, Bonfanti R, Sadler JE, Cramer EM, Chapman B and Mayadas TN. Induction of specific storage organelles by von Willebrand factor propolypeptide. Cell 64:403-413, 1991.

43. Erban JK and **Wagner DD.** A 130 kDa protein on endothelial cells binds to amino acids 15-42 of the Bβ chain of fibrinogen. J Biol Chem 267:2451-2458, 1992.

44. Mayadas TN and **Wagner DD.** Vicinal cysteines in the prosequence play a role in von Willebrand factor multimer assembly. Proc Natl Acad Sci USA 89:3531-3535, 1992.

45. Larsen GR, Sako D, Ahern TJ, Shaffer M, Erban JK, Sajer SA, Gibson RM, **Wagner DD,** Furie BC and Furie B. P-Selectin and E-Selectin: Distinct but overlapping leukocyte ligand specificities. J Biol Chem 267:11104-11110, 1992.

46. Koedam JA, Cramer EM, Briend E, Furie B, Furie BC and **Wagner DD**. P-selectin, a granule membrane protein of platelets and endothelial cells, follows the regulated secretory pathway in AtT-20 cells. J Cell Biol 116:617-625,1992.

47. Stone JP and **Wagner DD**. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. J Clin Invest 92:804-813, 1993.

48. Vischer UM and **Wagner DD**. CD63 is a component of Weibel-Palade bodies of human endothelial cells. Blood 82:1184-1191, 1993.

49. Subramaniam M, Koedam JA and **Wagner DD**. Divergent fates of P- and E-selectins after their expression on the plasma membrane. Molec Biol of the Cell 4:791-801, 1993.

50. Journet AM, Saffaripour S and **Wagner DD**. Requirement for both D domains of the propolypeptide in von Willebrand factor multimerization and storage. Thromb Haemost 70:1053-1057,1993.

51. Mayadas TN, Johnson RC, Rayburn H, Hynes RO and **Wagner DD**. Leukocyte rolling and extravasation are severely compromised in P-selectin-deficient mice. Cell 74:541-554, 1993.

52. **Wagner DD**. Weibel-Palade body: The storage granule for von Willebrand factor and P-selectin. State of the Art book, Thromb Haemost 70:105-110, 1993.

53. Journet AM, Saffaripour S, Cramer EM, Tenza D, and **Wagner DD**. von Willebrand factor storage requires intact prosequence cleavage site. Eur J Cell Biol 60:31-41, 1993.

54. Vischer UM and **Wagner DD**. von Willebrand factor proteolytic processing and multimerization precede the formation of Weibel-Palade bodies. Blood 83:3536-3544, 1994.

55. **Wagner DD** and Hynes RO. Gene targeting of adhesion molecules in the vasculature. Series: Molecular genetics and gene therapy of cardiovascular diseases. Mockrin SC (Ed.) New York: Marcel Dekker, pp. 403-425, 1995.

56. Stone JP and **Wagner DD**. P-selectin-deficient mice: A model to study inflammation and metastasis. In: Series: Topics in Molecular Medicine Vol 1. Siess W, Lorenz R and Weber PC. (Eds.). New York: Raven Press, pp. 105-116, 1995.

57. **Wagner DD**. P-selectin knock-out: a mouse model for various human diseases. In: Cell adhesion and human disease. Ciba Foundation Symposium 189. Marsh J and Goode J (Eds). Chichester: John Wiley & Sons, pp. 2-16, 1995.

58. **Wagner DD** and Ginsburg D. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 2nd Edition. Benz EJ, Cohen HJ, Furie B, Hoffman R and Shattil SJ. (Eds.). New York: Churchill Livingstone, 112:1717-1725, 1995.

59. Johnson RC, Mayadas TN, Frenette PS, Mebius RE, Subramaniam M, Lacasce A, Hynes RO and **Wagner DD**. Blood cell dynamics in P-selectin-deficient mice. Blood 86:1106-1114, 1995.

60. Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO and **Wagner DD**. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. J Exp Med 181:2277-2282, 1995.

61. **Wagner, DD**. P-selectin chases a butterfly. Editorial. J Clin Invest 95:1955-1956, 1995.

62. Frenette PS, Johnson RC, Hynes RO and **Wagner DD.** Platelets roll on stimulated endothelium *in vivo*: An interaction mediated by endothelial P-selectin. Proc Natl Acad Sci USA 92:7450-7454, 1995.

63. Yamada S, Mayadas TN, Yuan F, **Wagner DD,** Hynes RO, Melder RJ and Jain RK. Rolling in P-selectin deficient mice is reduced but not eliminated in the dorsal skin. Blood 86:3487-3492, 1995.

64. Pinsky DJ, Naka Y, Liao H, Oz MC, **Wagner DD,** Mayadas TN, Johnson RC, Hynes RO, Heath M, Lawson CA and Stern DM. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies: A mechanism for rapid neutrophil recruitment following cardiac preservation. J Clin Invest 97:493-500, 1996.

65. Mayadas TN, Mendrick DL, Brady HR, Tang T, Papayianni A, Assmann KJM, **Wagner DD,** Hynes RO and Cotran RS. Acute passive anti-glomerular basement membrane nephritis in P-selectin-deficient mice: Increased neutrophil influx and proteinuria and decreased lipoxin A_4 generation. Kidney Int 49:1342-1349,1996.

66. Subramaniam M, Frenette PS, Saffaripour S, Johnson RC, Hynes RO and **Wagner DD.** Defects in hemostasis in P-selectin-deficient mice. Blood 87:1238-1242, 1996.

67. Frenette PS, Mayadas TN, Rayburn H, Hynes RO and **Wagner DD.** Susceptibility to infection and altered hematopoiesis in mice deficient in both P-and E-selectins. Cell 84:563-574, 1996.

68. Tang T, Frenette PS, Hynes RO, **Wagner DD** and Mayadas TN. Cytokine-induced meningitis is dramatically attenuated in mice deficient in endothelial selectins. J Clin Invest 97:2485-2490, 1996.

69. Frenette PS and **Wagner DD.** Adhesion Molecules - Part I. New Engl J Med 334:1526-1529, 1996.

70. Frenette PS and **Wagner DD.** Adhesion Molecules - Part II: Blood vessels and blood cells. New Engl J Med 335:43-45, 1996.

71. Hynes RO and **Wagner DD.** Genetic manipulation of vascular adhesion molecules in mice. J Clin Invest 98:2193-2195, 1996.

72. Johnson RC, Chapman SM, Dong ZM, Ordovas JM, Mayadas TN, Herz J, Hynes RO, Schaefer EJ and **Wagner DD.** Absence of P-selectin reduces fatty streak formation in mice. J Clin Invest 99:1037-1043, 1997.

73. Subramaniam M, Saffaripour S, Van De Water L, Frenette PS, Mayadas TN, Hynes RO and **Wagner DD.** Role of endothelial selectins in wound repair. Am J Path 150:1701-1709, 1997.

74. Frenette PS and **Wagner DD.** Insights into selectin function from knockout mice. Thromb Haemost, State-of-the-Art Issue, 78:60-64, 1997.

75. Dong ZM, Gutierrez-Ramos JC, Coxon A, Mayadas TN and **Wagner DD.** A new class of obesity genes encodes leukocyte adhesion receptors. Proc Natl Acad Sci USA 94:7526-7530, 1997.

76. Walter UM, Ayer LM, Wolitzky BA, **Wagner DD,** Hynes RO, Manning AM and Issekutz AC. Characterization of a novel adhesion function blocking monoclonal antibody to rat/mouse P-selectin generated in the P-selectin-deficient mouse. Hybridoma 16: 249-257, 1997.

77. Walter UM, Ayer LM, Manning AM, Frenette PS, **Wagner DD**, Hynes RO, Wolitzky BA and Issekutz AC. Generation and characterization of a novel adhesion function blocking monoclonal antibody recognizing both rat and mouse E-selectin. Hybridoma 16: 355-361, 1997.

78. Weiser MR, Pechet TTV, Williams JP, Ma M, Frenette PS, Moore FD, Kobzk L, Hynes RO, **Wagner DD**, Carroll MC and Hechtman HB. Experimental murine acid aspiration injury is mediated by neutrophils and the alternative complement pathway. J Appl Physiol 83:1090-1095, 1997.

79. Dong ZM and **Wagner DD**. Leukocytes fighting against obesity. Molecular Psychiatry 3:8-9, 1998.

80. Frenette PS, Moyna C, Hartwell DW, Lowe JB, Hynes RO and **Wagner DD**. Platelet-endothelial interactions in inflamed mesenteric venules. Blood 91:1318-1324, 1998.

81. de Mora F, Williams CMM, Frenette PS, **Wagner DD**, Hynes RO, and Galli SJ. P- and E-selectins are required for the leukocyte recruitment, but not the tissue swelling, associated with IgE- and mast cell-dependent inflammation in mouse skin. Laboratory Investigation 78:497-505, 1998.

82. Fleming JC, Berger G, Guichard J, Cramer EM, and **Wagner DD**. The transmembrane domain enhances granular targeting of P-selectin. Euro J Cell Biol 75:331-343, 1998.

83. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, and **Wagner DD**. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest 102:145-152, 1998.

84. Denis C, Methia N, Frenette PS, Rayburn H, Ullman-Culleré M, Hynes RO, and **Wagner DD**. A mouse model of severe von Willebrand disease: Defects in hemostasis and thrombosis. Proc Natl Acad Sci USA 95:9524-9529, 1998.

85. Seiler KP, Ma Y, Weis JH, Frenette PS, Hynes RO, **Wagner DD**, and Weis JJ. E- and P-selectin are not required for resistance to severe murine Lyme arthritis. Infection and Immunity. 66:4557-2559, 1998.

86. Homeister JW, Zhang M, Frenette PS, Hynes RO, **Wagner DD**, Lowe JB, and Marks RM. Overlapping functions of E- and P-Selectin in neutrophil recruitment during acute inflammation. Blood. 92:2345-2352, 1998.

87. Hartwell D, Butterfield CE, Frenette PS, Kenyon BM, Hynes RO, Folkman J, and **Wagner DD**. Angiogenesis in P-and E-Selectin deficient mice. Microcirculation. 5:173-178, 1998.

88. Dong ZM and **Wagner DD**. Leukocyte-endothelial adhesion molecules in atherosclerosis. J Lab Clin Med 132:369-375, 1998.

89. Mazo IB, Gutierrez-Ramos JC, Frenette PS, Hynes RO, **Wagner DD**, and von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: Parallel contributions of endothelial selectins and VCAM-1. J Exp Med 18:465-474, 1998.

90. Berger G, Hartwell DW and **Wagner DD**. P-selectin and platelet clearance. Blood: 92:4446-4452, 1998.

91. Hartwell DW, Mayadas T, Frenette PS, Berger G, Rayburn H, Hynes RO and **Wagner DD**. Role of P-selectin cytoplasmic domain in granular targeting in vivo and in early inflammatory responses. J Cell Biol 143: 129-1141, 1998.

92. Frenette PS, Subbarao S, Mazo IB, von Andrian UH and **Wagner DD**. Endothelial selectins and VCAM-1 promote hematopoietic progenitor homing to bone marrow. Proc Natl Acad Sci USA 95:14423-14428, 1998.

93. Jia GQ, Gonzalo JA, Hidalgo A, **Wagner D**, Cybulsky M and Gutierrez-Ramos JC. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA4/VCAM-1 interactions. Int Immunol 11:1-10, 1999.

94. Shi C, Feinberg MW, Zhang D, Patel A, Sim CU, Dong ZM, Chapman SC, Gutierrez-Ramos JC, **Wagner DD**, Sibinga NES and Haber E. Donor MHC and Adhesion Molecules in Transplant Arteriosclerosis. J Clin Invest 103:469-474, 1999.

95. Soriano SG, Wang YF, **Wagner DD** and Frenette PS. P- and E-selectin -deficient mice are susceptible to cerebral ischemia-reperfusion injury. Brain Res 835:360-364, 1999.

96. Robinson SD, Frenette PS, Rayburn H, Cummiskey M, Ullman-Culleré M, **Wagner DD**, and Hynes RO, Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. Proc Natl Acad Sci USA 96:11452-11457, 1999.

97. Enjyoji K, Sévigny J, Lin Y, Frenette PS, Christie PD, Schulte am Esch J, Imai M, Edelberg JM, Rayburn H, Lech M, Beeler DL, Csizmadia E, **Wagner DD**, Robson SC and Rosenberg RD. Targeted disruption of *cd39*/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nature Med 5: 1010-1017, 1999.

98. Hartwell, DW and **Wagner DD**. New discoveries with mice mutant in endothelial and platelet selectins. Thromb Haemost, State-of-the-Art Issue, 82:850-857, 1999.

99. Denis C and **Wagner DD**. Insights from von Willebrand disease animal models. Cell Mol Life Sci 56: 977-990, 1999.

100. Methia N, Denis C, and **Wagner DD**. Carboxypeptidase E does not mediate von Willebrand factor targeting to storage granules. Euro J Cell Biol 78:884-891, 1999.

101. Ginsburg D and **Wagner DD**. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 3rd Edition. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE and McGlave P. (Eds.). New York: Churchill Livingstone, 1999.

102. Dong ZM, Brown AA and **Wagner DD**. The prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. Circulation 101:2290-2295, 2000.

103. Frenette PS, Denis CV, Weiss L, Subbarao S, Hartwig JH, Vestweber D and **Wagner DD**. P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. J Exp Med 191:1413-1422, 2000.

104. Ni H, Denis CV, Subbarao S, Degen JL, Sato TN, Hynes RO and **Wagner DD**. Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. J Clin Invest 106:385-392, 2000.

105. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM and **Wagner DD**. Platelets adhere and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood 96:3322-3328, 2000.

106. Andre P, Hartwell D, Hrachovinova I, Saffaripour S and **Wagner DD**. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. Proc Natl Acad Sci USA 97:13835-13840, 2000.

107. Denis CV, Kwack K, Saffaripour S, Maganti S, Andre P, Schaub RG and **Wagner DD**. Interleukin-11 significantly increases plasma von Willebrand factor and factor VIII in wild-type and von Willebrand disease mouse models. Blood 97:465-472, 2001.

108. Denis CV, André P, Saffaripour S and **Wagner DD**. Defect in regulated secretion of P-selectin leukocyte recruitment in von Willebrand factor-deficient mice. Proc Natl Acad Sci USA, 98:4072-4077, 2001.

109. Niederman R, Westernoff T, Lee C, Mark LL, Kawashima N, Ullman-Culler M, Dewhirst FE, Paster BJ, **Wagner DD**, Mayadas T, Hynes RO and Stashenko P. Infection-mediated early-onset periodontal disease in P/E-selectin-deficient mice. J Clin Periodontal 28:569-575, 2001.

110. Ni H, Ramakrishnan V, Ruggeri ZM, Papalia JM, Phillips DR and **Wagner DD**. Increased thrombogenesis and embolus formation in mice lacking glycoprotein V. Blood 98:368-373, 2001.

111. Methia N, André P, Denis CV, Economopoulos M and **Wagner DD**. Localized reduction of atherosclerosis in von Willebrand factor-deficient mice. Blood 98:1424-1428, 2001.

112. Methia N, André P, Hafezi-Moghadam A, Economopoulos M, Thomas KL and **Wagner DD**. ApoE deficiency compromises the blood brain barrier especially after injury. Mol Med 7:810-815, 2001.

113. Gotte M, Joussen AM, Klein C, Andre P, **Wagner DD**, Hinkes MT, Kirchhof B, Adamis AP, and Bernfield M. Role of syndecan-1 in leukocyte-endothelial interactions in the ocular vasculature. Invest Ophthalmol Vis Sci 43:1135-1141, 2002.

114. André P, Prasad KSS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR and **Wagner DD**. CD40L stabilizes arterial thrombi by a β3 integrin-dependent mechanism. Nature Med. 8:247-252, 2002.

115. Sethi S, Ziouzenkova O, Ni H, **Wagner DD**, Plutzky J and Mayadas TN. Oxidized omega-3 fatty acids in fish oil inhibit leukocyte-endothelial interactions through activation of PPARα. Blood 100:1340-1346, 2002.

116. Hoffmeister KM, Felbinger TW, Falet H, Denis CV, Bergmeier W, Mayadas TN, von Andrian UH, **Wagner DD**, Stossel TP and Hartwig JH. The clearance mechanism of chilled blood platelets. Cell 112: 87-97, 2003.

117. Ni H, Yuen PST, Papalia JM, Trevithick JE, Sakai T, Fässler R, Hynes RO and **Wagner DD**. Plasma fibonectin promotes thrombus growth and stability in injured arterioles. Proc Natl Acad Sci USA 100: 2415-2419, 2003.

118. Burger PC and **Wagner DD**. Platelet P-selectin facilitates atherosclerotic lesion development. Blood 101:2661-2666, 2003.

119. Italiano JE Jr, Bergmeier W, Tiwari S, Falet H, Hartwig JH, Hoffmeister K, André P, **Wagner DD** and Shivdasani RA. Mechanisms and implications of platelet discoid shape. Blood 101:4789-96, 2003.

120. Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian HH Jr, Schaub RG, McEver RP and **Wagner DD**. P-selectin/PSGL-1 interaction generates microparticles that correct hemostasis in a murine model of hemophilia A. Nat Med 9:1020-1025, 2003.

121. Matsushita K, Morrell CN, Cambien B, Yang SX, Yamakuchi M, Bao C, Hara M, Quick RA, Cao W, O'Rourke B, Lowenstein JM, Pevsner J, **Wagner DD** and Lowenstein CJ. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. Cell 115:1-20, 2003.

122. **Wagner DD.** Shear madness in TNF-α signaling. Blood 102:2711-2712, 2003.

123. Myers DD, Hawley AE, Farris DM, Wrobelski SK, Thanaporn P, Schaub RG, **Wagner DD**, Kumar A and Wakefield TW. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg 38: 1075-1089, 2003.

124. Ni H, Papalia JM, Degen JL and **Wagner DD.** Control of thrombus embolization and fibronectin internalization by integrin αIIbβ3 engagement of the fibrinogen γ chain. Blood 102: 3609-3614, 2003.

125. Bergmeier W, Burger PC, Piffath CL, Hoffmeister KM, Hartwig JH, Nieswandt B and **Wagner DD.** Metalloproteinase inhibitors improve the recovery and hemostatic function of in vitro-aged or -injured mouse platelets. Blood 102: 4229-4235, 2003.

126. Cambien B, Bergmeier W, Saffaripour S, Mitchell HA and **Wagner DD.** Anti-thrombotic activity of TNF-α. J Clin Invest 112: 1589-1596, 2003.

127. **Wagner DD** and Burger PC. Platelets in inflammation and thrombosis. Arterioscler Thromb Vasc Biol 23: 2131-2137, 2003.

128. Cambien B and **Wagner DD.** A new role in hemostasis for the adhesion receptor P-selectin. Trends Mol Med 10: 179-186, 2004.

129. Bergmeier W, Rabie T, Strehl A, Piffath CL, Prostredna M, **Wagner DD** and Nieswandt B. GPVI down-regulation in murine platelets through metalloproteinase-dependent shedding. Thromb Haemost 91: 951-958. 2004.

130. Kondo T, Hafezi-Moghadam A, Thomas K, **Wagner DD** and Kahn CR. Mice lacking insulin or insulin-like growth factor 1 receptors in vascular endothelial cells maintain normal blood-brain barrier. Biochem Biophys Res Commun 317: 315-320, 2004.

131. Crittenden JR, Bergmeier W, Zhang Y, Piffath CL, Liang Y, **Wagner DD**, Housman DE and Graybiel AM. CalDAG-GEFI integrates signaling for platelet aggregation and thrombus formation. Nat Med 10: 982-986, 2004.

132. Bergmeier W, Piffath CL, Cheng G, Dole VS, Zhang Y, von Andrian UH and **Wagner DD.** Tumor necrosis factor-α-converting enzyme (ADAM17) mediates GPIbα shedding from platelets in vitro and in vivo. Circ Res 95: 677-683, 2004.

133. Ginsburg D and **Wagner DD.** Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 4th Edition. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE and McGlave P. (Eds.). New York: Churchill Livingstone, 2004.

134. Babic AM, Wang HW, Lai MJ, Daniels TG, Felbinger TW, Burger PC, Stricker-Krongrad A and **Wagner DD.** ICAM-1 and β2 integrin deficiency impairs fat oxidation and insulin metabolism during fasting. In press, Mol Med.

135. Wang, HW, Babic, AM, Mitchell HA, Liu K and **Wagner DD.** Elevated soluble ICAM-1 levels induce immune deficiency and increase adiposity in mice. In press, FASEB J.

RELATED PROCEEDINGS APPENDIX

None

Attorney Docket No. CFBF-P02-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant(s):	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	October 10, 1997		
For:	METHOD FOR TREATING OR INHIBITING ATHEROSCLEROSIS WITH PSGL-1		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Mail Stop Appeals, on 1/3/06.

Patricia McKenney

Mail Stop Appeal
Commissioner for Patents
P.O. 1450
Alexandria, VA. 22313-1450
ATTENTION: Board of Patent Appeals and Interferences

Sir:

APPELLANT'S BRIEF ON APPEAL

This is an appeal to the Board of Patent Appeals and Interferences (the "Board") from the decision of the Examiner finally rejecting claims 71, 81, 85, 87-89, 92 and 94-97, and is in furtherance of the Notice of Appeal filed on November 3, 2005, in this application. The appealed claims are as set forth in the attached Claims Appendix. Provision for the payment of fees required for filing this brief, and any required extension of time for filing the brief, are submitted herewith. This brief is submitted in triplicate in accordance with the provisions of 37 C.F.R. §1.192(a).

REAL PARTY IN INTEREST

The real party in interest in this appeal is the CBR Institute for Biomedical Research, Inc., aka The Center for Blood Research, Inc., the assignee of the rights of the inventors in the above-identified patent application. The CBR Institute for Biomedical Research, Inc., is an affiliate of the Harvard Medical School.

RELATED APPEALS AND INTERFERENCES

There are believed to be no related appeals or interferences that will directly affect, be directly affected by, or have a bearing on the Board's decision in this appeal.

STATUS OF CLAIMS

The status of the claims in this application is as follows. Claims 71, 81, 85, 87-89, 92 and 94-97 are pending and are on appeal. Claims 1-70, 72-80, 82-84, 86, 90, 91 and 93 have been canceled. The limitations in claims 71 and 95 directed to mimetics of PSGL-1 has been withdrawn from consideration. Claims 71 and 95 are independent claims. Claim 71 is directed to methods for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on the arterial walls of a mammal. Claim 95 is directed to a method for treating atherosclerosis in a mammal which has been subjected to a vessel corrective technique.

STATUS OF AMENDMENTS

Claims 71, 81, 85, 87-89, 92 and 94-97 were finally rejected in the final Office Action of May 3, 2005. A Notice of Appeal was filed on November 3, 2005. No amendments have been made or entered following issuance of the final Office Action.

SUMMARY OF CLAIMED SUBJECT MATTER

Atherosclerosis is a principal cause of heart attacks among adults in the United States. This condition results from the restricted flow of arterial blood due to the accumulation of fibrous plaque over time in the arterial lumen. Death or incapacity of the subject may result from the rupture of the fibrous cap of the plaque, causing hemorrhage, thrombosis and occlusion of the artery. The fibrous plaque is formed from fatty streaks which develop into lesions composed predominantly of layers of smooth muscle cells, lipid-filled macrophages, and T cells. The earliest stages of atherosclerosis occur when migrating monocytes and T lymphocytes bind to the lumen of the arterial wall. Atherosclerosis is a chronic, long term condition, and is distinguished from more acute conditions such as local inflammation. Page 1, line 17 to page 2, line 22. Appellants have found that P-selectin is implicated in the origins of atherosclerosis as a result of the mediation of platelet or endothelial cell binding and adhesion to monocytes.

In one embodiment of the invention, as described in claim 71, the invention is directed to a method for treating or inhibiting atherosclerosis in a mammal by decreasing the formation or growth of plaque on arterial walls in a mammal. This is accomplished by administering to the mammal an agent selected from the group consisting of PSGL-1, soluble forms of PSGL-1, and fragments of PSGL-1. The agent is administered to the mammal in repeated sequential doses or by controlled release methods over a period of months or years, and is effective to inhibit the interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin. Page 3, lines 1-16; page 4, lines 23-28; page 5, lines 6-14 and 27-32; page 7, lines 1-3; and page 12, line 21 to page 14, line 2.

In another embodiment, as described in claim 95, the invention is directed to a method for treating atherosclerosis in a mammal by performing a vessel corrective technique on a mammal selected form the group consisting of angioplasty, stenting, atherectomy and bypass surgery on the mammal, and subsequently administering to the mammal an effective amount of an agent selected form the group consisting of PSGL-1, soluble forms of PSGL-1, and PSGL-1 fragments. The administration of the agent is accomplished over a period of months or years, and results in a decrease in the formation or growth of plaque on the arterial walls. Page 13, line 21 to page 14, line 2; and page 14, lines 20-29.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether claims 71, 81, 85, 87-89, 92 and 94-97 are unpatentable under 35 U.S.C. 103(a) as obvious over Cummings et al. (U.S. Patent No. 5,464,778) in view of Larsen et al. (U.S. Patent No. 5,840,679), Tedder et al. (U.S. Patent No. 5,834,425), Coller et al. (U.S. Patent No. 5,976,532), Sluiter et al. (*J. Cardiovascular Pharmacology*, 22 (Suppl. 4): S37-S44 (1993)), Aberg et al. (U.S. patent No. 5,061,694), Casscells et al. (U.S. Patent No. 5,308,622), Hinstridge et al. (*Drugs*, 42 (Suppl. 2): 8-2 (1991)), *The Merck Manual of Diagnosis and Therapy*, 16th Ed., pages 409-413 (1992), and De Felice et al. (*Angiology* 41:1-11 (1990))

2. Whether claims 71, 81, 85, 87-89, 92 and 94-97 are unpatentable based on the judicially created doctrine of obviousness-type double patenting in view of claims 40-41, 49-52, 59-60 and 73 of U.S. Patent Application No. 09/436,076.

ARGUMENTS

I. Rejection of Claims 71, 81, 85, 87-89, 92 and 94-97 as obvious over Cummings et al. in view of Larsen et al., Tedder et al., Coller et al., Sluiter et al., Aberg et al., Casscells et al., Hinstridge et al., *The Merck Manual of Diagnosis and Therapy*, and De Felice et al.

The primary reference cited by the Examiner, Cummings et al., discusses atherosclerosis in a section labeled "Clinical Applications". See col. 18, line 33 of the patent. In particular, the Cummings, et al. patent states that atherosclerosis is an example of a pathological condition in which an inflammatory response may occur, and that the P-selectin glycoprotein ligand can be used to treat such an inflammatory responses. See col. 18, lines 34-53 of the reference.

It is appellants' position that Cummings et al. is directed to the treatment of acute inflammatory conditions, such as ischemica and reperfusion, rather than atherosclerosis. The focal point of the reference is the prevention of leukocyte adherence to vascular endothelium. With regard to atherosclerosis, the reference makes the following comments, at col. 19, line 64 to col. 20, line 5:

> "Platelet-leukocyte interactions are believed to be important in atherosclerosis. Platelets might have a role in recruitment of monocytes into atherosclerotic plaques; the

accumulation of monocytes is known to be one of the earliest detectable events during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia."

These comments do not teach or suggest that the reference contemplates the use of PSGL-1 for reducing the formation of arterial plaque. Rather, the reference is directing one skilled in the art to the treatment of thrombus (blood clot) formation. Such treatment would involve the prevention of platelet activation by leukocytes as described elsewhere in the reference. A reduction is plaque formation is not inherent in the treatment of a thrombosis since plaque reduction would require a treatment regime of months or years.

Furthermore, Cummings et al. is directed to the prevention of platelet activation in the circulatory system, rather that the inhibition of endothelial cell binding which is an essential component of atherosclerosis. See, in particular, the Wagner (II)132 Declaration, at paragraphs 4, 5 and 6. Thus, it is appellants' position that one skilled in the art, reading the Cummings et al. reference, would have no reasonable expectation that PSGL-1 could be used to reduce plaque formation, and further, that a long term treatment regime would be required to achieve this result.

Appellants also respectfully submit that the Cummings, et al. reference has been antedated as a result of the prior conception and subsequent reduction to practice of the claimed invention, coupled with the requisite diligence, as shown in the Wagner 131 Declaration.

Larsen et al., like Cummings et al., does not relate to the treatment of chronic conditions, such as atherosclerosis, but is instead directed to the treatment of inflammatory or acute conditions. Contrary to the position taken in the Office Action, neither Cummings et al. nor Larsen et al. disclose that a treatment for atherosclerosis can be administered in conjunction with a vessel-corrective technique.

Coller et al. relates to the treatment of a thrombotic condition using antibodies to GPIIb/IIIa. The present invention, in contrast, relates to the use of PSGL-1, and variants therefore, rather than antibodies. Cummings et al. does not teach the use of vessel corrective techniques, and does not teach the use of antibodies for therapeutic purposes. Consequently, applicants maintain that there is no basis for combining the Coller et al. and Cummings et al. references.

The Sluiter et al. reference has been cited to provide further evidence that one skilled in the art would have targeted the inhibition of P-selectin-mediated events for inhibiting leukocyte adhesion receptors to alleviate tissue damage in cardiovascular diseases. However, although the Sluiter et al. reference mentions P-selectin in a general sense, there is no disclosure in the reference concerning the inhibition of P-selectin binding to the ligand of P-selectin. In fact, the Sluiter et al. reference is actually directed to the possible role of oxygen-derived free radicals in the treatment of inflammation. See the Summary portion of the reference on page S37, and the discussion on page S38. Accordingly, the Sluiter et al. reference does not oversome the shortcomings of Cummings et al.

The remaining references cited by the Examiner do not cure the deficiencies of the Cummings et al. Larsen et al., Coller et al. and Sluiter et al. references as discussed above. references. In particular, the Aberg et al., Casscells et al. and Hinstridge et al. references do not relate to the use of appellant's agent for the treatment of diseases. Accordingly, it would be entirely speculative to suggest that the use of appellants' particular agents for the treatment of atherosclerosis can be administered over a prolonged period of time, and that such treatment would have beneficial results.

Similarly, the Merck and De Felice et al. references are apparently relied upon for linking atherosclerosis with a decrease in plaque growth or formation. Of course, appellants do not claim to have discovered the scientific basis for atherosclerosis. Rather , appellants have developed a treatment protocol for atherosclerosis which is not disclosed or suggested in any of the cited references.

Finally, appellants note that the sheer number of references required for formulating the present obviousness rejection (a total of 10 references) is itself a strong indication that the present claims are not obvious.

II. Rejection of Claims 71, 81, 85, 87-89, 92 and 94-97 as unpatentable based on obviousness-type double patenting in view of claims 40-41, 49-52, 59-60 and 73 of U.S. Patent Application No. 09/436,076).

U.S. Patent Application No. 09/436,076, which forms the basis of this rejection, has now been abandoned. Accordingly, this rejection is now moot.

Summarizing, for the reasons presented in this brief, appellants respectfully urge the Board to reverse the rejection made in the Final Office Action, and to allow all of the appended claims.

Appellants hereby authorize the Commissioner, to debit the $500.00 fee for filing this appeal brief from Appellant's Deposit Account No. 18-1945. If there are any other fees not accounted for above, Appellants hereby authorize the Commissioner to charge the fee to Deposit Account 18-1945.

Respectfully submitted,
GOSZ AND PARTNERS

Date: 01/03/06

William Gosz

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CLAIMS APPENDIX

71. A method for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on arterial walls in a mammal comprising:

providing an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, said P-selectin being on an endothelial cell; and

administering said agent to a mammal in need of such treatment so as to cause such inhibition to occur, said agent being administered repeatedly in sequential doses or by the controlled release to the mammal over a period of months or years,

wherein said agent is selected from the group consisting of PSGL-1, soluble forms of PSGL-1, fragments of PSGL-1, and mimetics of PSGL-1 which resemble PSGL-1 in shape and charge distribution, said agent being effective to inhibit the interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin.

81. The method of claim 71 wherein said P-selectin can bind to said PSGL-1 in the absence of said agent.

85. The method of claim 71 wherein said agent is administered in combination with other therapeutic agents.

87. The method of claim 71 wherein said mammal is human.

88. The method of claim 71 wherein said agent is administered in a dose of from about 0.01 mg/kg to about 200mg/kg of body weight.

89. The method of claim 71 wherein said agent is administered at a dose of about 100 mg/kg of body weight.

92. The method of claim 95, wherein said vessel-corrective technique is selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery.

94. The method of claim 95, wherein said agent is administered in combination with other therapeutic agents.

95. A method for treating atherosclerosis in a mammal to which a vessel-corrective technique is administered comprising:

performing a vessel-corrective technique selected form the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery on a mammal; and

administering to said mammal, after said vessel-corrective technique, an effective amount of an agent selected from the group consisting of PSGL-1, soluble forms of PSGL-1, fragments of PSGL-1, and mimetics of PSGL-1 which resemble PSGL-1 in shape and charge distribution, said agent being administered repeatedly in sequential doses or by the controlled release to the mammal over a period of months or years to decrease the formation or growth of plaque on the arterial walls of the mammal.

96. The method of claim 71 wherein the agent is administered over a period of years.

97. The method of claim 95 wherein the agent is administered over a period of years.

EVIDENCE APPENDIX

1. Declaration of Denisa Wagner and Robert Johnson Under Rule 131*

2. Declaration of Denisa Wagner under Rule 132**

* Considered and entered by the Examiner on March 18, 2003.

** Considered and entered by the Examiner on February 25, 2005.

Ex. No. 1

ATTORNEY DOCKET NO. CFBF-P03-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wagner et al.
Examiner: P. Gambel
Serial No.: 08/436,076
Art Unit: 1644
Filing Date: November 8, 1999
For: METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Washington, D.C. 20231 on 3/12/03.

Patricia McKenney

BOX AMENDMENT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231
Dear Sir:

DECLARATION UNDER 37 CFR 1.131

We, Denisa D. Wagner and Robert C. Johnson, declare and state as follows:

1. We are the applicants of the above-identified patent application, and the co-inventors of the subject matter disclosed and claimed therein.

2. We are familiar with the present claims of the above-identified application, which are directed to methods for treating or inhibiting atherosclerosis in a mammal by administering an agent that inhibits an interaction between P-selectin and PSGL-1 and E-selectin and a ligand of E-selectin., e.g. PSGL-1 (P-selectin glycoprotein ligand-1), soluble forms of PSGL-1, fragments of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

3. We conceived the claimed invention at least as early as 1988, and coupled with due diligence from a time prior to November 16, 1992, reduced the claimed in invention to practice at least as early as May 6, 1994.

4. Exhibit A is a copy of a page showing a note authored by co-inventor Denisa Wagner in 1988. The notes shown in the Exhibit were recorded by Dr. Wagner during the conference of the American Heart Association held in 1988, and were written on the last page of the program booklet next to a listing of meetings to be held in 1989. The note on the bottom right hand side of the page states that

> Macrophages (Mφ) eat bits of activated platelets. ELAM-1 = Padgem. Do monocytes bind to Padgem on platelets. Padgem is an opsomizing agent to get rid of debris of platelets.

The term "Padgem" here refers to P-selectin and the term "ELAM-1" refers to E-selectin (Endothelial Leukocyte Adhesion Molecule). In 1988, E-selectin was known to mediate endothelial binding to leukocytes. We conceived that there is a functional relationship between E-selectin and P-selectin, and that P-selectin mediates the binding of platelets to macrophages (leukocytes implicated in atherosclerosis).By binding to Padgem, the macrophages are "eating" bits of activated platelets, thereby increasing the fat (lipid) content of the macrophages, and promoting their conversion into foam cells (macrophage cells with a "foamy" appearance due to the presence of lipids that act as precursors for atherosclerotic plaque). Exhibit A thus demonstrates that we had identified a role for P-selectin and E-selectin in some of the key pathological events involved in atherosclerosis, e.g. macrophage binding to P-selectin on platelets, from a time well before November 16, 1992.

5. Exhibit B, also written by Dr. Wagner, describes an experiment we conceived on February 28, 1992. Exhibit B states:

> Breed P-selectin deficient mouse with a mouse strain that develops atherosclerosis. See if it (atherosclerosis) can be prevented.

According to this proposed experiment, a mouse deficient in P-selectin would be bred with a mouse strain that develops atherosclerosis to determine whether atherosclerosis can be prevented. In other words, we conceived that if P-selectin/ligand binding and/or E-selectin/ligand binding could be inhibited *in vivo* in a mammal, the atherosclerotic lesions could be reduced or inhibited. In order to complete this experiment, we understood that it would first be necessary to prepare a P-selectin knock-out mouse, and breed this mouse with mouse strains susceptible to atherosclerosis. It is known that mice are generally resistant to developing atherosclerosis. The mouse strain most susceptible to developing atherosclerosis is the C57 black mouse. But the C57 mouse must still be fed a high lipid diet to observe any meaningful development of atherosclerosis.

6. Exhibit C, also written by Dr. Wagner, describes a proposal we conceived on March 2, 1992, to study the role of the P-selectin in atherosclerosis by developing a suitable mouse model, and feeding the P-selectin deficient mice (mutants) and control wild-type mice (P-selectin positive) with a lipid diet. The, formation of atherosclerotic lesions in the mice would be studied and characterized. Exhibit C states, on page 5:

> Study the role of P-selectin in atherosclerosis by feeding P-selectin deficient and P-selectin positive mice a lipid diet. Study the formation of atherosclerotic lesions in mice.

Page 5 of Exhibit C also poses the question whether von Willebrand (vW) disease pigs may be resistant to atherosclerosis because of a lack of P-selectin. P-selectin is stored in granules containing vW factor, and these granules are absent in vW disease.

7. At a time prior to November 16, 1992, we undertook to prepare a mouse model for subsequent testing. The mouse model took at least 4 years to prepare, and was completed on or about September 13, 1993. In order to prepare the mouse model, we used a knock-out mouse deficient in P-selectin and back-crossed this mouse with C57 black mice. In order to be sure that the resulting mutant mouse would be susceptible to atherosclerotic lesion development, we

decided to breed 4 generations of mice, with each generation being more susceptible to atherosclerosis. First we developed a P-selectin deficient mouse. Then we bred the P-selectin deficient mouse with a C57 black mouse. Finally, we bred the offspring of the first breeding with another C57 black mouse, and so on for a total of 4 back-cross breedings. We reasoned that the fourth generation would be suitable for evaluation. It took us about 3 years to make a P-selectin deficient mouse, and another year to complete the back-crossing process with the C57 black mice. This work was laborious and continuous, and consumed a large amount of our time and effort. Although the general technology for creating mouse models had been developed by others, we were the first to develop a P-selectin-deficient mouse model. We diligently worked on successfully constructing such a model, and verifying the correct properties and characteristics of the mutant mouse by about September 13, 1993.

8. After the preparation of the mutant mouse deficient in P-selectin on the C57 black background, we promptly commenced feeding the mice (control and experimental) a diet high in lipids. The experimental and control mice were fed a lipid diet for approximately eight months prior to sacrificing the animals and recording the data, This took approximately 8 months since even the C57 black mice are somewhat resistant to the formation of atherosclerosis. Immediately thereafter, we sacrificed the animals and evaluated them for the size and character of atherosclerotic lesions. We prepared the table enclosed as Exhibit D on May 6, 1994. The table in Exhibit D shows the size of atherosclerotic lesions in P-deficient (mutant) mice compared to wild type mice as controls. These results demonstrate a reduction in the size of atherosclerotic lesions in P-selectin deficient mice. Based on these results we concluded that inhibitors of P-selectin/ligand binding and/or E-selectin/ligand binding would be useful for the treatment or inhibition of atherosclerosis, and this constitutes an actual reduction to practice of the claimed invention.

9. From the above information, we deduced that inhibitors of P-selectin and/or E-selectin could be used to treat atherosclerosis in mammals based on the role of P-selectin and/or E-selectin on the pathogenesis of atherosclerosis as presently claimed in the above-identified application. We further believe that the above information constitutes evidence the claimed

invention was conceived prior to November 16, 1992, and diligently reduced to practice at least as early as the actual reduction to practice date of May 6, 1994.

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/25/2003
Date

Denisa D. Wagner

3-6-03
Date

Robert C. Johnson

AMERICAN HEART ASSOCIATION CME OFFERINGS

1989 Highlights

For information contact the American Heart Association, Scientific Sessions, 7320 Greenville Avenue, Dallas, Texas 75231.

***SCIENTIFIC CONFERENCE ON MEMBRANE EVENTS AND INTRACELLULAR SIGNALLING IN THE CARDIOVASCULAR SYSTEM**
Waikoloa, Hawaii
AHA Council on Basic Science and the Japanese Heart Foundation
January 7-11, 1989
Conference Chairman: James T. Stull, PhD

14TH INTERNATIONAL JOINT CONFERENCE ON STROKE AND CEREBRAL CIRCULATION
San Antonio, TX
AHA Council on Stroke
February 9-11, 1989
Conference Chairman: Vladimir C. Hachinski, MD

SCIENTIFIC CONFERENCE ON CORONARY ATHEROSCLEROSIS AND THROMBOSIS
Keystone, CO
AHA Councils on Circulation, Atherosclerosis, Thrombosis, and Clinical Cardiology
February 22-25, 1989
Conference Chairman: Paul J. Cannon, MD

2ND INTERNATIONAL CONFERENCE ON PREVENTIVE CARDIOLOGY AND THE ANNUAL MEETING OF THE AHA COUNCIL ON EPIDEMIOLOGY
Washington, DC
AHA Council on Epidemiology
June 18-22, 1989
Conference Chairman: Jeremiah Stamler, MD

***15TH TEN-DAY SEMINAR ON THE EPIDEMIOLOGY AND PREVENTION OF CARDIOVASCULAR DISEASES**
Tahoe City, CA
AHA Council on Epidemiology
July 30-August 12, 1989
Conference Chairman: Darwin R. Labarthe, MD, PhD

43RD ANNUAL FALL CONFERENCE AND SCIENTIFIC SESSIONS OF THE COUNCIL FOR HIGH BLOOD PRESSURE RESEARCH
Cleveland, OH
AHA Council for High Blood Pressure Research
September 26-29, 1989
Conference Chairman: Allen W. Cowley, Jr, PhD

62ND SCIENTIFIC SESSIONS
New Orleans, LA
AHA Scientific Councils
November 13-16, 1989
Conference Chairman: Michael R. Rosen, MD

*Limited attendance

do plt release by ↓ blocks w ^{125}β inhibit B/β 15-42

put flow through of ϕ column to on to fibrin column. ϕ rec. should be reduced. control do it reverse go back to fibrin clots does after reacting w fibrin the receptor get phosphorylated

Put on column EC grown in *Phosphate I stimulation w fibrin, EDTA elute If this works to A23 release ϕ incubation etc.
Do x-linking - it will work

Add ϕ to cell lysate put on fibrin column. ϕ should inhibit IIB IIIa-like binding but fibrin specific b. should not be affected !!

elute w RGD, b. to fibrin may not be through RGD or elute w γ pept.

see if severe vWd plts plts have Padgem

---

m ϕ eat bits of activated plts ELAM-1 ! = Padgem

do monocytes b. to Padgem on plts Padgem is an opsonizing agent to get rid of pieces of plts

A23187 makes these vesicles. are these same as Padgem ?

feb. 28/92

Bread ⊖P-ed mouse with a mouse strain that develops atherosclerosis see if it can be prevented.

Projects for Bob

3/2/92

Prepare antibodies to P-s. cytoplasmic tail (polyclonal). Do they recognise other granular proteins → clone them

Schaffhausen: Abs to SH2 domain of PDGF rec. binds to other prot. containing this homologous domain

Role of dibasic cleavage site in targeting to storage granules

Insulin c-DNA is available that has both sites mutated. When expressed in AtT-20 cells will it be stored?

Randy Kaufman has a protein inhibitor of PACE (and likely related enzymes. It could be transfected into cells and see if storage is prevented (ACTH, vWf etc).

In endothelial cells that do not express vWf — what happens to P-sel

a) culture EC in the presence

Role of vicinal cysteines in integrins matrix assembly?

Targeting of P-selectin in yeast

Is there a storage compartment in yeast

use yeast secretion mutants and clathrin ⊖ cells to find the cellular machinery responsible for targeting of transmembrane proteins.

In the absence of vWf in EC, what happens to P-sel?

a) use vWdisease pigs EC
does our Ab x-react?
disadvantage: availability
advantage - normal platelets: vWd plts

b) use #UVEC grown in the presence of antisense oligos to vWf
→ inhibit vWf synthesis
see where P-sel is and if it can be transported to cell surface
+/- secretagogues

vWdisease pigs are resistant to atherosclerosis. Is this an effect of vWf (lack of) or P-selectin absence?

Study role of P-S. in atherosclerosis
by feeding ⊖ P-S and ⊕ P-S lipid diet to mice → formation of atherosclerotic lesions

5/6/94 Denisa Bob

	Genotype	Score
271	Mut	-
278	WT	++. 2.7
279	Mut	-
137	Mut	-
119	WT	+ 0.5
548 E	Mut	+ 0.2
269	WT	++
270	Mut	+ small
40	WT	+++
34	Mut	-
35	WT	+
19	WT	+ (Maybe 2+)
20	WT	2+
268	WT	+++
42	Mut	-
106	WT	3+++
18	Mut	-
89	WT	-

Rachelle A. Rosenbaum 5/9/94
City of Boston
Suffolk County
a true copy of the original

MY COMMISSION EXPIRES JUNE 6, 1997

** See when it is deep lesion & not raised it is not a lesion. #34

#19 Must be positive about lesion.

Not good

EXHIBIT D
Page 1 of 1

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Ex. No. 2

Attorney Docket No. CFBF-P02-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant(s):	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	November 8, 1999		
For:	METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS WITH PSGL-1		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, P.O. 1450, Alexandria, VA. 22313-1450 on February 24 2005.

Patricia McKenney

Commissioner for Patents
P.O. 1450
Alexandria, VA. 22313-1450

DECLARATION UNDER RULE 132

Sir:

I, Denisa D. Wagner, declare and state as follows:

1. I am a Professor in the Department of Pathology at Harvard Medical School, and a Senior Investigator at The CBR Institute for Biomedical Research, Inc., Boston, Massachusetts. My Curriculum Vitae is attached hereto as an Exhibit. I am also an inventor of the above-identified patent application. I consider myself to be an expert in the field of cardiovascular medicine and pathology, as reflected in my Curriculum Vitae, and I am well aware of the knowledge level of others skilled in this art.

2. I have reviewed the outstanding Office Action of October 21, 2004, in the above-identified patent application. I am also familiar with the claims of this application, as presently amended, which are directed to methods for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on arterial walls. This is accomplished by administering PSGL-1, or selected variants thereof, to a subject over a prolonged period of time, i.e. months or years.

3. I am familiar with the references cited by the Examiner in the outstanding Office Action. In particular, I have reviewed the Cummings et al. reference (U.S. Patent No. 5,464,778), which I understand to be the primary reference cited in the Office Action.

4. The Cummings et al. reference is generally directed to inflammatory thrombotic conditions such as ischemia and reperfusion. In col. 19, line 64 to col. 20, line 5, the Cummings et al. reference makes the following disclosure relating to atherosclerosis and platelet-leukocyte interactions:

> "Platelet-leukocyte interactions are believed to be important in atherosclerosis. Platelets might have a role in recruitment of monocytes into atherosclerotic plaques; the accumulation of monocytes is known to be one of the earliest detectable events during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia."

5. My interpretation of the above cited passage is as follows. Cummings et al. speculate that platelet-leukocyte interactions are important in atherosclerosis. In fact, it is now well established that the key in atherosclerotic lesion development is the direct binding of monocytes to endothelial cells, and the reference does not discuss this. Cummings et al. discusses events following plaque rupture. Such events include thrombus formation leading to ischemic injury causing neutrophil recruitment. This event occurs long after plaque formation which is subject of the present application. The claims of our application specify that the P-selectin is on endothelial cells. Endothelial cells coat the arterial wall, and are not part of the

circulatory system as are the platelets. The plaque rupture, thrombotic events and neutrophil recruitment to the ischemic area discussed in the reference are not part of the present application.

6. I believe that the present invention can be distinguished from the Cummings et al. reference in the following respects. The present invention is directed to the treatment of atherosclerosis by decreasing the formation or growth of plaque on arterial walls. Atherosclerosis is a chronic condition caused by many factors, primarily by excessive plasma cholesterol levels, and results in the deposition of lesions and plaque on arterial walls. The treatment of atherosclerosis requires the long term administration of a medication to a subject in order to result in a meaningful improvement of the condition. This contrast with the treatment of a thrombosis, as disclosed in the Cummings et al. reference, which requires the commencement of an immediate treatment regime in order to prevent the reoccurrence of a thrombotic attack.

7. I also believe that the ability to design a mimetic of PSGL-1 having similar inhibitory characteristics, i.e. the ability to inhibit P-selectin, would be within the skill of a person in the art. Such a mimetic would optimally be designed based on a similarity of charge and shape as stated in the present claims.

8. Based on my knowledge, training and experience, it is my opinion that the references cited in the outstanding Office Action would not teach or suggest the method for treating atherosclerosis as stated in the present claims of the above-identified patent application.

I further declare that statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 2/18/05

Denisa D. Wagner, Ph.D.

CURRICULUM VITAE

DENISA D. WAGNER, Ph.D.

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Boston, MA 02115
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PLACE OF BIRTH: Prague, Czechoslovakia; U.S. citizen

EDUCATION: Universite de Geneve, Switzerland - Biochemistry
Diploma of Biochemistry, 1975, with distinction

Massachusetts Institute of Technology, Cambridge, MA
Biology - Ph.D., 1980

Harvard University, Cambridge, MA
M.A. (honorary), 1997

FACULTY POSITIONS:

Professor of Pathology, Harvard Medical School, Boston, MA.
1997-present.

Senior Investigator, The CBR Institute for Biomedical Research (formerly known as The Center for Blood Research), Boston, MA.
1994-present.

Associate Professor of Pathology, Harvard Medical School, Boston, MA.
1994-1997.

Associate Professor of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA. 1989-1994.

Associate Professor of Medicine, Tufts University School of Medicine and Member, Special and Scientific Staff, New England Medical Center, Boston, MA. 1987-1994.

Assistant Professor of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1985-1987.

Assistant Professor of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1982-1987.

Senior Instructor in Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1981-1982.

AWARDS:

Established Investigator Award, American Heart Association, Biosynthesis of von Willebrand protein by endothelial cells. 1986-1991.

XIth ISTH Congress award in recognition of an outstanding communication, 1987.

Gwendolyn J. Stewart Memorial Award to honor women in the biomedical sciences, 1998.

Special recognition award from the Council on Arteriosclerosis, Thrombosis and Vascular Biology, AHA, 1998

MERIT award, National Heart, Lung and Blood Institute, NIH, 1998-2008.

Sol Sherry Lecture, American Heart Association, 2004.

MAJOR COMMITTEE ASSIGNMENTS:

University:

1991-1994	Member of the Graduate Advisory Committee of the Graduate Program in Cell, Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University
1992-1994	Sackler School Committee on Programs and Faculty, Tufts University
1992-1994	Graduate Admission Committee of the Graduate Program in Cell, Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University
1995-Present	Member of the Graduate Program in Biological and Biomedical Sciences, Harvard Medical School
1998-Present	Member, Committee for Immunology, Program in Immunology, Harvard Medical School
1999-2002	Member of the Faculty Fellowship Committee, Harvard Medical School
2001-2004	Member, Standing Committee on Promotions, Reappointments, and Appointments, Harvard Medical School
2003-Present	Elected Member, Harvard Medical School Faculty Council

National and Regional:

Served on many review committees and panels for the National Institutes of Heath, American Heart Association, Juvenile Diabetes Foundation and American Red Cross.

Currently permanent member, NIH, NHLBI Thrombosis and Hemostasis Study Section (2002-2006)

MEMBERSHIPS, OFFICES, AND COMMITTEE ASSIGNMENTS IN PROFESSIONAL SOCIETIES:

1980-Present	American Society for Cell Biology
1982-Present	American Society of Hematology
1982-Present	International Society of Thrombosis and Haemostasis
1983-1997	Council on Thrombosis, American Heart Association
1985-Present	International Society of Thrombosis and Haemostasis, subcommittee on von Willebrand factor
1991-1996	American Heart Association, Vascular Wall Biology Study Committee

1992-Present	American Heart Association, Council on Thrombosis Executive Committee
1993-1995	American Heart Association, Council on Thrombosis Long-Range Planning Committee
1994-1996	American Heart Association, Council on Thrombosis Membership Committee (Chairman)
1994-Present	American Association for the Advancement of Science
1994-Present	North American Vascular Biology Organization (Founding Member)
1995-1998	American Society of Hematology, Scientific Subcommittee on Thrombosis & Vascular Biology
1997-Present	North American Vascular Biology Organization (Councilor)
1997-Present	Council on Arteriosclerosis, Thrombosis and Vascular Biology, American Heart Association (Fellow)
1998	Council of the Gordon Research Conferences (Member)
1998-Present	The Molecular Medicine Society (Member)
1999-Present	Boston Obesity Nutrition Research Center (Member)
2001-Present	National Hemophilia Foundation (Member)
2001-2005	American Society of Hematology, Scientific Committee on Thrombosis & Vascular Biology (Member)
2002-Present	Harvard Center for Neurodegeneration & Repair (Member)
2004-2010	Council of the International Society on Thrombosis and Haemostasis (Member)
2004-2005	Chair, Scientific Committee on Thrombosis and Vascular Biology, American Society of Hematology

EDITORIAL BOARDS:

1993-2004	Molecular Biology of the Cell
1994-1999	Journal of Clinical Investigation
1998-Present	Molecular Medicine
2003-2008	Blood

PUBLICATIONS
DENISA D. WAGNER, Ph.D.

1. Hynes RO, Ali IU, Destree AT, Mautner V, Perkins ME, Senger DR, **Wagner DD** and Smith KK. A large glycoprotein lost from the surfaces of transformed cells. Ann NY Acad Sci 312:317-342, 1978.

2. **Wagner DD** and Hynes RO. Domain structure of fibronectin and its relation to function (disulfides and sulfhydryl groups). J Biol Chem 254:6746-6754, 1979.

3. Hynes RO, Destree AT, Perkins ME and **Wagner DD.** Cell surface fibronectin and oncogenic transformation. J Supramolecular Str 11:95-104, 1979.

4. **Wagner DD** and Hynes RO. Topological arrangement of the major structural features of fibronectin. J Biol Chem 255:4304-4312, 1980.

5. **Wagner DD,** Ivatt R, Destree AT and Hynes RO. Similarities and differences between fibronectins of normal and transformed hamster cells. J Biol Chem 256:11708-11715, 1981.

6. **Wagner DD** and Hynes RO. Fibronectin coated beads are endocytosed by cells and align with microfilament bundles. Exp Cell Res 140:373-381, 1982.

7. Van De Water L III, **Wagner DD,** Crenshaw EB III and Hynes RO. Fibronectin-dependent endocytosis by macrophage-like ($P388D_1$) and fibroblastic (NIL 8) cells. In: Cellular Recognition. Glaser L, Frazier W and Gottlieb D (Eds.). New York: Alan R. Liss, 869-878, 1982.

8. Hynes RO, Destree AT and **Wagner DD.** Relationships between microfilaments, cell-substratum adhesion and fibronectin. Cold Spring Harbor Symposia on Quantitative Biology 46:659-669, 1982.

9. **Wagner DD,** Olmsted JB and Marder VJ. Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. J Cell Biol 95:355-360, 1982.

10. **Wagner DD** and Marder VJ. Biosynthesis of von Willebrand protein by human endothelial cells: identification of a large precursor polypeptide chain. J Biol Chem 258:2065-2067, 1983.

11. **Wagner DD,** Urban-Pickering M and Marder VJ. von Willebrand protein binds to extracellular matrices independently of collagen. Proc Natl Acad Sci USA 81:471-475. 1984.

12. Sporn LA, Rubin P, Marder VJ and **Wagner DD.** Irradiation induces release of von Willebrand protein from endothelial cells in culture. Blood 64:567-570, 1984.

13. **Wagner DD** and Marder VJ. Biosynthesis of von Willebrand protein by human endothelial cells: processing steps and their intracellular localization. J Cell Biol 99:2123-2130, 1984.

14. Sporn LA, Chavin SI, Marder VJ and **Wagner DD.** Biosynthesis of von Willebrand protein by human megakaryocytes. J Clin Invest 76:1102-1106, 1985.

15. **Wagner DD,** Mayadas T, Urban-Pickering M, Lewis BH and Marder VJ. Inhibition of disulfide bonding of von Willebrand protein by monensin results in small, functionally defective multimers. J Cell Biol 101:112-120, 1985.

16. Fay PJ, Kawai Y, **Wagner DD,** Ginsberg D, Bonthron D, Ohlsson-Wilhelm BM, Chavin SI, Abraham GN, Handin RI, Orkin SH, Montgomery RR and Marder VJ. Propolypeptide of von Willebrand factor circulates in blood and is identical to von Willebrand antigen II. Science 232:995-998, 1986.

17. Sporn LA, Marder VJ and **Wagner DD.** Inducible secretion of large biologically potent von Willebrand factor multimers. Cell 46:185-190, 1986.

18. **Wagner DD,** Mayadas T and Marder VJ. Initial glycosylation and acidic pH in the Golgi apparatus are required for multimerization of von Willebrand factor. J Cell Biol 102:1320-1324, 1986.

19. **Wagner DD,** Lawrence SO, Ohlsson-Wilhelm BM, Fay PJ and Marder VJ. Topology and order of formation of interchain disulfide bonds in von Willebrand factor. Blood 69:27-32, 1987.

20. **Wagner DD,** Fay PJ, Sporn LA, Sinha S, Lawrence SO and Marder VJ. Divergent fates of von Willebrand factor and its propolypeptide (von Willebrand antigen II) after secretion from endothelial cells. Proc Natl Acad Sci USA 84:1955-1959, 1987.

21. Sporn LA, Marder VJ and **Wagner DD.** von Willebrand factor released from Weibel-Palade bodies binds more avidly to extracellular matrix than that secreted constitutively. Blood 69:1531-1534, 1987.

22. Sinha S and **Wagner DD.** Intact microtubules are necessary for complete processing, storage and regulated secretion of von Willebrand factor by endothelial cells. Eur J Cell Biol 43:377-383, 1987.

23. Ribes JA, Francis CW and **Wagner DD.** Fibrin induces release of von Willebrand factor from endothelial cells. J Clin Invest 79:117-123, 1987.

24. Mayadas TN, **Wagner DD** and Simpson PJ. von Willebrand factor biosynthesis and partitioning between constitutive and regulated pathways of secretion after thrombin stimulation. Blood 73:706-711, 1989.

25. Handin RI and **Wagner DD.** von Willebrand factor: molecular and cellular biology. In: Progress in Hemostasis and Thrombosis (vol. 9). Coller B (Ed.). Philadelphia: W B Saunders, 233-259, 1989.

26. Roarke MC, **Wagner DD,** Marder VJ and Sporn LA. Temperature-sensitive steps in the secretory pathway for von Willebrand factor in endothelial cells. Eur J Cell Biol 48:337-343, 1989.

27. Sporn LA, Marder VJ and **Wagner DD.** Differing polarity of the constitutive and regulated secretory pathways for von Willebrand factor in endothelial cells. J Cell Biol 108:1283-1289, 1989.

28. Bonfanti R, Furie BC, Furie B and **Wagner DD.** PADGEM (GMP-140) is a component of Weibel-Palade bodies of human endothelial cells. Blood 73:1109-1112, 1989.

29. Avissar N, Whitin JC, Allen PZ, **Wagner DD,** Liegey P and Cohen HJ. Plasma selenium dependent glutathione peroxidase: cell of origin and secretion. J Biol Chem 264:15850-15855, 1989.

30. Ribes JA, Ni F, **Wagner DD** and Francis CW. Mediation of fibrin-induced release of von Willebrand factor from cultured endothelial cells by the fibrin β chain. J Clin Invest 1989, 84:435-442, 1989.

31. Mayadas TN and **Wagner DD.** In vitro multimerization of von Willebrand factor is triggered by low pH: importance of the propolypeptide and free sulfhydryls. J Biol Chem 264:13497-13503, 1989.

32. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, **Wagner DD** and Furie B. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 59:305-312, 1989.

33. **Wagner DD.** Storage and secretion of von Willebrand factor. In: Role of Factor VIII and von Willebrand Factor. Zimmerman TS and Ruggeri ZM. (Eds.). New York: Marcel Dekker, pp. 161-180, 1989.

34. Larsen E, Palabrica T, Sajer S, Gilbert GE, **Wagner DD,** Furie BC and Furie B. PADGEM-dependent adhesion of activated platelets to monocytes and neutrophils is mediated by a lineage-specific carbohydrate, lacto-N-fucopentaose III (CD15). Cell 63:467-474, 1990.

35. **Wagner DD.** Cell biology of von Willebrand factor. In: Annual Review of Cell Biology. Palade GE, Alberts BM and Spudich JS. (Eds). 6:217-246, 1990.

36. Peters JH, Sporn LA, Ginsberg MH and **Wagner DD.** Human endothelial cells synthesize, process, and secrete fibronectin molecules bearing an alternatively spliced type III homology (ED1). Blood 75:1801-1808, 1990.

37. Mayadas TN and **Wagner DD.** von Willebrand factor biosynthesis and processing. Ann NY Acad Sci, 614:153-166, 1991.

38. Furie B, Celi A, Palabrica TM, Larsen E, **Wagner DD** and Furie, BC. PADGEM a Leukocyte Receptor on Activated Platelets: Biology and application to in vivo medical diagnostics. In: Biotechnology of Plasma Proteins: Haemostasis, Thrombosis and Iron Proteins. Albertini A, Lenfant CL, Mannucci PM, and Sixma JJ. (Eds). Basel: Karger, 32-36, 1991.

39. **Wagner DD** and Bonfanti R. von Willebrand factor and the endothelium. Mayo Clinic Proceedings 66:621-627, 1991.

40. Bevilacqua E, Butcher B, Furie B, Furie B, Gallatin M, Gimbrone M, Harlan J, Kishimoto K, Lasky L, McEver R, Paulson J, Rosen S, Seed B, Springer T, Stoolman L, Tedder T, Varki A, **Wagner DD**, Weissman I and Zimmerman G. Letter to the Editor. Selectins: A family of adhesion receptors. Cell 67:233, 1991.

41. **Wagner DD.** Structure and biology of von Willebrand factor. In: Hematology: Basic Principles and Practices. Benz EJ, Cohen HJ, Furie B, Hoffman R and Shattil SJ. (Eds.). New York: Churchill Livingstone, 1354-1358, 1991.

42. **Wagner DD,** Saffaripour S, Bonfanti R, Sadler JE, Cramer EM, Chapman B and Mayadas TN. Induction of specific storage organelles by von Willebrand factor propolypeptide. Cell 64:403-413, 1991.

43. Erban JK and **Wagner DD.** A 130 kDa protein on endothelial cells binds to amino acids 15-42 of the Bβ chain of fibrinogen. J Biol Chem 267:2451-2458, 1992.

44. Mayadas TN and **Wagner DD.** Vicinal cysteines in the prosequence play a role in von Willebrand factor multimer assembly. Proc Natl Acad Sci USA 89:3531-3535, 1992.

45. Larsen GR, Sako D, Ahern TJ, Shaffer M, Erban JK, Sajer SA, Gibson RM, **Wagner DD**, Furie BC and Furie B. P-Selectin and E-Selectin: Distinct but overlapping leukocyte ligand specificities. J Biol Chem 267:11104-11110, 1992.

46. Koedam JA, Cramer EM, Briend E, Furie B, Furie BC and **Wagner DD**. P-selectin, a granule membrane protein of platelets and endothelial cells, follows the regulated secretory pathway in AtT-20 cells. J Cell Biol 116:617-625,1992.

47. Stone JP and **Wagner DD**. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. J Clin Invest 92:804-813, 1993.

48. Vischer UM and **Wagner DD**. CD63 is a component of Weibel-Palade bodies of human endothelial cells. Blood 82:1184-1191, 1993.

49. Subramaniam M, Koedam JA and **Wagner DD**. Divergent fates of P- and E-selectins after their expression on the plasma membrane. Molec Biol of the Cell 4:791-801, 1993.

50. Journet AM, Saffaripour S and **Wagner DD**. Requirement for both D domains of the propolypeptide in von Willebrand factor multimerization and storage. Thromb Haemost 70:1053-1057,1993.

51. Mayadas TN, Johnson RC, Rayburn H, Hynes RO and **Wagner DD**. Leukocyte rolling and extravasation are severely compromised in P-selectin-deficient mice. Cell 74:541-554, 1993.

52. **Wagner DD**. Weibel-Palade body: The storage granule for von Willebrand factor and P-selectin. State of the Art book, Thromb Haemost 70:105-110, 1993.

53. Journet AM, Saffaripour S, Cramer EM, Tenza D, and **Wagner DD**. von Willebrand factor storage requires intact prosequence cleavage site. Eur J Cell Biol 60:31-41, 1993.

54. Vischer UM and **Wagner DD**. von Willebrand factor proteolytic processing and multimerization precede the formation of Weibel-Palade bodies. Blood 83:3536-3544, 1994.

55. **Wagner DD** and Hynes RO. Gene targeting of adhesion molecules in the vasculature. Series: Molecular genetics and gene therapy of cardiovascular diseases. Mockrin SC (Ed.) New York: Marcel Dekker, pp. 403-425, 1995.

56. Stone JP and **Wagner DD**. P-selectin-deficient mice: A model to study inflammation and metastasis. In: Series: Topics in Molecular Medicine Vol 1. Siess W, Lorenz R and Weber PC. (Eds.). New York: Raven Press, pp. 105-116, 1995.

57. **Wagner DD**. P-selectin knock-out: a mouse model for various human diseases. In: Cell adhesion and human disease. Ciba Foundation Symposium 189. Marsh J and Goode J (Eds). Chichester: John Wiley & Sons, pp. 2-16, 1995.

58. **Wagner DD** and Ginsburg D. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 2nd Edition. Benz EJ, Cohen HJ, Furie B, Hoffman R and Shattil SI. (Eds.). New York: Churchill Livingstone, 112:1717-1725, 1995.

59. Johnson RC, Mayadas TN, Frenette PS, Mebius RE, Subramaniam M, Lacasce A, Hynes RO and **Wagner DD**. Blood cell dynamics in P-selectin-deficient mice. Blood 86:1106-1114, 1995.

60. Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO and **Wagner DD**. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. J Exp Med 181:2277-2282, 1995.

61. **Wagner, DD**. P-selectin chases a butterfly. Editorial. J Clin Invest 95:1955-1956, 1995.

62. Frenette PS, Johnson RC, Hynes RO and **Wagner DD.** Platelets roll on stimulated endothelium *in vivo*: An interaction mediated by endothelial P-selectin. Proc Natl Acad Sci USA 92:7450-7454, 1995.

63. Yamada S, Mayadas TN, Yuan F, **Wagner DD,** Hynes RO, Melder RJ and Jain RK. Rolling in P-selectin deficient mice is reduced but not eliminated in the dorsal skin. Blood 86:3487-3492, 1995.

64. Pinsky DJ, Naka Y, Liao H, Oz MC, **Wagner DD,** Mayadas TN, Johnson RC, Hynes RO, Heath M, Lawson CA and Stern DM. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies: A mechanism for rapid neutrophil recruitment following cardiac preservation. J Clin Invest 97:493-500, 1996.

65. Mayadas TN, Mendrick DL, Brady HR, Tang T, Papayianni A, Assmann KJM, **Wagner DD,** Hynes RO and Cotran RS. Acute passive anti-glomerular basement membrane nephritis in P-selectin-deficient mice: Increased neutrophil influx and proteinuria and decreased lipoxin A_4 generation. Kidney Int 49:1342-1349,1996.

66. Subramaniam M, Frenette PS, Saffaripour S, Johnson RC, Hynes RO and **Wagner DD.** Defects in hemostasis in P-selectin-deficient mice. Blood 87:1238-1242, 1996.

67. Frenette PS, Mayadas TN, Rayburn H, Hynes RO and **Wagner DD.** Susceptibility to infection and altered hematopoiesis in mice deficient in both P-and E-selectins. Cell 84:563-574, 1996.

68. Tang T, Frenette PS, Hynes RO, **Wagner DD** and Mayadas TN. Cytokine-induced meningitis is dramatically attenuated in mice deficient in endothelial selectins. J Clin Invest 97:2485-2490, 1996.

69. Frenette PS and **Wagner DD.** Adhesion Molecules - Part 1. New Engl J Med 334:1526-1529, 1996.

70. Frenette PS and **Wagner DD.** Adhesion Molecules - Part II: Blood vessels and blood cells. New Engl J Med 335:43-45, 1996.

71. Hynes RO and **Wagner DD.** Genetic manipulation of vascular adhesion molecules in mice. J Clin Invest 98:2193-2195, 1996.

72. Johnson RC, Chapman SM, Dong ZM, Ordovas JM, Mayadas TN, Herz J, Hynes RO, Schaefer EJ and **Wagner DD.** Absence of P-selectin reduces fatty streak formation in mice. J Clin Invest 99:1037-1043, 1997.

73. Subramaniam M, Saffaripour S, Van De Water L, Frenette PS, Mayadas TN, Hynes RO and **Wagner DD.** Role of endothelial selectins in wound repair. Am J Path 150:1701-1709, 1997.

74. Frenette PS and **Wagner DD.** Insights into selectin function from knockout mice. Thromb Haemost, State-of-the-Art Issue, 78:60-64, 1997.

75. Dong ZM, Gutierrez-Ramos JC, Coxon A, Mayadas TN and **Wagner DD.** A new class of obesity genes encodes leukocyte adhesion receptors. Proc Natl Acad Sci USA 94:7526-7530, 1997.

76. Walter UM, Ayer LM, Wolitzky BA, **Wagner DD,** Hynes RO, Manning AM and Issekutz AC. Characterization of a novel adhesion function blocking monoclonal antibody to rat/mouse P-selectin generated in the P-selectin-deficient mouse. Hybridoma 16: 249-257, 1997.

77. Walter UM, Ayer LM, Manning AM, Frenette PS, **Wagner DD**, Hynes RO, Wolitzky BA and Issekutz AC. Generation and characterization of a novel adhesion function blocking monoclonal antibody recognizing both rat and mouse E-selectin. Hybridoma 16: 355-361, 1997.

78. Weiser MR, Pechet TTV, Williams JP, Ma M, Frenette PS, Moore FD, Kobzk L, Hynes RO, **Wagner DD**, Carroll MC and Hechtman HB. Experimental murine acid aspiration injury is mediated by neutrophils and the alternative complement pathway. J Appl Physiol 83:1090-1095, 1997.

79. Dong ZM and **Wagner DD**. Leukocytes fighting against obesity. Molecular Psychiatry 3:8-9, 1998.

80. Frenette PS, Moyna C, Hartwell DW, Lowe JB, Hynes RO and **Wagner DD**. Platelet-endothelial interactions in inflamed mesenteric venules. Blood 91:1318-1324, 1998.

81. de Mora F, Williams CMM, Frenette PS, **Wagner DD**, Hynes RO, and Galli SJ. P- and E-selectins are required for the leukocyte recruitment, but not the tissue swelling, associated with IgE- and mast cell-dependent inflammation in mouse skin. Laboratory Investigation 78:497-505, 1998.

82. Fleming JC, Berger G, Guichard J, Cramer EM, and **Wagner DD**. The transmembrane domain enhances granular targeting of P-selectin. Euro J Cell Biol 75:331-343, 1998.

83. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, and **Wagner DD**. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest 102:145-152, 1998.

84. Denis C, Methia N, Frenette PS, Rayburn H, Ullman-Culleré M, Hynes RO, and **Wagner DD**. A mouse model of severe von Willebrand disease: Defects in hemostasis and thrombosis. Proc Natl Acad Sci USA 95:9524-9529, 1998.

85. Seiler KP, Ma Y, Weis JH, Frenette PS, Hynes RO, **Wagner DD**, and Weis JJ. E- and P-selectin are not required for resistance to severe murine Lyme arthritis. Infection and Immunity. 66:4557-2559, 1998.

86. Homeister JW, Zhang M, Frenette PS, Hynes RO, **Wagner DD**, Lowe JB, and Marks RM. Overlapping functions of E- and P-Selectin in neutrophil recruitment during acute inflammation. Blood. 92:2345-2352, 1998.

87. Hartwell D, Butterfield CE, Frenette PS, Kenyon BM, Hynes RO, Folkman J, and **Wagner DD**. Angiogenesis in P-and E-Selectin deficient mice. Microcirculation. 5:173-178, 1998.

88. Dong ZM and **Wagner DD**. Leukocyte-endothelial adhesion molecules in atherosclerosis. J Lab Clin Med 132:369-375, 1998.

89. Mazo IB, Gutierrez-Ramos JC, Frenette PS, Hynes RO, **Wagner DD**, and von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: Parallel contributions of endothelial selectins and VCAM-1. J Exp Med 18:465-474, 1998.

90. Berger G, Hartwell DW and **Wagner DD**. P-selectin and platelet clearance. Blood: 92:4446-4452, 1998.

91. Hartwell DW, Mayadas T, Frenette PS, Berger G, Rayburn H, Hynes RO and **Wagner DD**. Role of P-selectin cytoplasmic domain in granular targeting in vivo and in early inflammatory responses. J Cell Biol 143: 129-1141, 1998.

92. Frenette PS, Subbarao S, Mazo IB, von Andrian UH and **Wagner DD**. Endothelial selectins and VCAM-1 promote hematopoietic progenitor homing to bone marrow. Proc Natl Acad Sci USA 95:14423-14428, 1998.

93. Jia GQ, Gonzalo JA, Hidalgo A, **Wagner D**, Cybulsky M and Gutierrez-Ramos JC. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA4/VCAM-1 interactions. Int Immunol 11:1-10, 1999.

94. Shi C, Feinberg MW, Zhang D, Patel A, Sim CU, Dong ZM, Chapman SC, Gutierrez-Ramos JC, **Wagner DD**, Sibinga NES and Haber E. Donor MHC and Adhesion Molecules in Transplant Arteriosclerosis. J Clin Invest 103:469-474, 1999.

95. Soriano SG, Wang YF, **Wagner DD** and Frenette PS. P- and E-selectin -deficient mice are susceptible to cerebral ischemia-reperfusion injury. Brain Res 835:360-364, 1999.

96. Robinson SD, Frenette PS, Rayburn H, Cummiskey M, Ullman-Culleré M, **Wagner DD**, and Hynes RO, Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. Proc Natl Acad Sci USA 96:11452-11457, 1999.

97. Enjyoji K, Sévigny J, Lin Y, Frenette PS, Christie PD, Schulte am Esch J, Imai M, Edelberg JM, Rayburn H, Lech M, Beeler DL, Csizmadia E, **Wagner DD**, Robson SC and Rosenberg RD. Targeted disruption of *cd39*/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nature Med 5: 1010-1017, 1999.

98. Hartwell, DW and **Wagner DD**. New discoveries with mice mutant in endothelial and platelet selectins. Thromb Haemost, State-of-the-Art Issue, 82:850-857, 1999.

99. Denis C and **Wagner DD**. Insights from von Willebrand disease animal models. Cell Mol Life Sci 56: 977-990, 1999.

100. Methia N, Denis C, and **Wagner DD**. Carboxypeptidase E does not mediate von Willebrand factor targeting to storage granules. Euro J Cell Biol 78:884-891, 1999.

101. Ginsburg D and **Wagner DD**. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 3rd Edition. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE and McGlave P. (Eds.). New York: Churchill Livingstone, 1999.

102. Dong ZM, Brown AA and **Wagner DD**. The prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. Circulation 101:2290-2295, 2000.

103. Frenette PS, Denis CV, Weiss L, Subbarao S, Hartwig JH, Vestweber D and **Wagner DD**. P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. J Exp Med 191:1413-1422, 2000.

104. Ni H, Denis CV, Subbarao S, Degen JL, Sato TN, Hynes RO and **Wagner DD**. Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. J Clin Invest 106:385-392, 2000.

105. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM and **Wagner DD**. Platelets adhere and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood 96:3322-3328, 2000.

106. Andre P, Hartwell D, Hrachovinova I, Saffaripour S and **Wagner DD**. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. Proc Natl Acad Sci USA 97:13835-13840, 2000.

107. Denis CV, Kwack K, Saffaripour S, Maganti S, Andre P, Schaub RG and **Wagner DD**. Interleukin-11 significantly increases plasma von Willebrand factor and factor VIII in wild-type and von Willebrand disease mouse models. Blood 97:465-472, 2001.

108. Denis CV, André P, Saffaripour S and **Wagner DD**. Defect in regulated secretion of P-selectin leukocyte recruitment in von Willebrand factor-deficient mice. Proc Natl Acad Sci USA, 98:4072-4077, 2001.

109. Niederman R, Westernoff T, Lee C, Mark LL, Kawashima N, Ullman-Culler M, Dewhirst FE, Paster BJ, **Wagner DD**, Mayadas T, Hynes RO and Stashenko P. Infection-mediated early-onset periodontal disease in P/E-selectin-deficient mice. J Clin Periodontal 28:569-575, 2001.

110. Ni H, Ramakrishnan V, Ruggeri ZM, Papalia JM, Phillips DR and **Wagner DD**. Increased thrombogenesis and embolus formation in mice lacking glycoprotein V. Blood 98:368-373, 2001.

111. Methia N, André P, Denis CV, Economopoulos M and **Wagner DD**. Localized reduction of atherosclerosis in von Willebrand factor-deficient mice. Blood 98:1424-1428, 2001.

112. Methia N, André P, Hafezi-Moghadam A, Economopoulos M, Thomas KL and **Wagner DD**. ApoE deficiency compromises the blood brain barrier especially after injury. Mol Med 7:810-815, 2001.

113. Gotte M, Joussen AM, Klein C, Andre P, **Wagner DD**, Hinkes MT, Kirchhof B, Adamis AP, and Bernfield M. Role of syndecan-1 in leukocyte-endothelial interactions in the ocular vasculature. Invest Ophthalmol Vis Sci 43:1135-1141, 2002.

114. André P, Prasad KSS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR and **Wagner DD**. CD40L stabilizes arterial thrombi by a β3 integrin-dependent mechanism. Nature Med. 8:247-252, 2002.

115. Sethi S, Ziouzenkova O, Ni H, **Wagner DD**, Plutzky J and Mayadas TN. Oxidized omega-3 fatty acids in fish oil inhibit leukocyte-endothelial interactions through activation of PPARα. Blood 100:1340-1346, 2002.

116. Hoffmeister KM, Felbinger TW, Falet H, Denis CV, Bergmeier W, Mayadas TN, von Andrian UH, **Wagner DD**, Stossel TP and Hartwig JH. The clearance mechanism of chilled blood platelets. Cell 112: 87-97, 2003.

117. Ni H, Yuen PST, Papalia JM, Trevithick JE, Sakai T, Fässler R, Hynes RO and **Wagner DD**. Plasma fibonectin promotes thrombus growth and stability in injured arterioles. Proc Natl Acad Sci USA 100: 2415-2419, 2003.

118. Burger PC and **Wagner DD**. Platelet P-selectin facilitates atherosclerotic lesion development. Blood 101:2661-2666, 2003.

119. Italiano JE Jr, Bergmeier W, Tiwari S, Falet H, Hartwig JH, Hoffmeister K, André P, **Wagner DD** and Shivdasani RA. Mechanisms and implications of platelet discoid shape. Blood 101:4789-96, 2003.

120. Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian HH Jr, Schaub RG, McEver RP and **Wagner DD**. P-selectin/PSGL-1 interaction generates microparticles that correct hemostasis in a murine model of hemophilia A. Nat Med 9:1020-1025, 2003.

121. Matsushita K, Morrell CN, Cambien B, Yang SX, Yamakuchi M, Bao C, Hara M, Quick RA, Cao W, O'Rourke B, Lowenstein JM, Pevsner J, **Wagner DD** and Lowenstein CJ. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. Cell 115:1-20, 2003.

122. **Wagner DD.** Shear madness in TNF-α signaling. Blood 102:2711-2712, 2003.

123. Myers DD, Hawley AE, Farris DM, Wrobelski SK, Thanaporn P, Schaub RG, **Wagner DD**, Kumar A and Wakefield TW. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg 38: 1075-1089, 2003.

124. Ni H, Papalia JM, Degen JL and **Wagner DD**. Control of thrombus embolization and fibronectin internalization by integrin αIIbβ3 engagement of the fibrinogen γ chain. Blood 102: 3609-3614, 2003.

125. Bergmeier W, Burger PC, Piffath CL, Hoffmeister KM, Hartwig JH, Nieswandt B and **Wagner DD**. Metalloproteinase inhibitors improve the recovery and hemostatic function of in vitro-aged or -injured mouse platelets. Blood 102: 4229-4235, 2003.

126. Cambien B, Bergmeier W, Saffaripour S, Mitchell HA and **Wagner DD**. Anti-thrombotic activity of TNF-α. J Clin Invest 112: 1589-1596, 2003.

127. **Wagner DD** and Burger PC. Platelets in inflammation and thrombosis. Arterioscler Thromb Vasc Biol 23: 2131-2137, 2003.

128. Cambien B and **Wagner DD**. A new role in hemostasis for the adhesion receptor P-selectin. Trends Mol Med 10: 179-186, 2004.

129. Bergmeier W, Rabie T, Strehl A, Piffath CL, Prostredna M, **Wagner DD** and Nieswandt B. GPVI down-regulation in murine platelets through metalloproteinase-dependent shedding. Thromb Haemost 91: 951-958. 2004.

130. Kondo T, Hafezi-Moghadam A, Thomas K, **Wagner DD** and Kahn CR. Mice lacking insulin or insulin-like growth factor 1 receptors in vascular endothelial cells maintain normal blood-brain barrier. Biochem Biophys Res Commun 317: 315-320, 2004.

131. Crittenden JR, Bergmeier W, Zhang Y, Piffath CL, Liang Y, **Wagner DD**, Housman DE and Graybiel AM. CalDAG-GEFI integrates signaling for platelet aggregation and thrombus formation. Nat Med 10: 982-986, 2004.

132. Bergmeier W, Piffath CL, Cheng G, Dole VS, Zhang Y, von Andrian UH and **Wagner DD**. Tumor necrosis factor-α-converting enzyme (ADAM17) mediates GPIbα shedding from platelets in vitro and in vivo. Circ Res 95: 677-683, 2004.

133. Ginsburg D and **Wagner DD**. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 4th Edition. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE and McGlave P. (Eds.). New York: Churchill Livingstone, 2004.

134. Babic AM, Wang HW, Lai MJ, Daniels TG, Felbinger TW, Burger PC, Stricker-Krongrad A and **Wagner DD**. ICAM-1 and β2 integrin deficiency impairs fat oxidation and insulin metabolism during fasting. In press, Mol Med.

135. Wang, HW, Babic, AM, Mitchell HA, Liu K and **Wagner DD**. Elevated soluble ICAM-1 levels induce immune deficiency and increase adiposity in mice. In press, FASEB J.

RELATED PROCEEDINGS APPENDIX

None

TRANSMITTAL OF APPEAL BRIEF

Docket No.: CFBF-P02-002

In re Application of: Wagner, Denisa

Application No.	Filing Date	Examiner	Group Art Unit
08/948,393	10/10/97	Phillip Gambel	1644

Invention: METHOD FOR TREATING OR INHIBITING ATHEROSCLEROSIS WITH PSGL-1

TO THE COMMISSIONER OF PATENTS:

Transmitted herewith is the Reply Brief in this application, with respect to the Office Action mailed from the PTO on May 3, 2005 and the Notice of Appeal filed on November 3, 2005.

The fee for filing this Appeal Brief is $500.00 .

[x] Large Entity [ ] Small Entity

[ ] A petition for extension of time is also enclosed.

The fee for the extension of time is _____ .

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Dated: January 3, 2006

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I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: MS Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

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Attorney Docket No. CFBF-P02-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant(s):	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	October 10, 1997		
For:	METHOD FOR TREATING OR INHIBITING ATHEROSCLEROSIS WITH PSGL-1		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Mail Stop Appeals, on 1/3/06.

Patricia McKenney

Mail Stop Appeal
Commissioner for Patents
P.O. 1450
Alexandria, VA. 22313-1450
ATTENTION: Board of Patent Appeals and Interferences

Sir:

APPELLANT'S BRIEF ON APPEAL

This is an appeal to the Board of Patent Appeals and Interferences (the "Board") from the decision of the Examiner finally rejecting claims 71, 81, 85, 87-89, 92 and 94-97, and is in furtherance of the Notice of Appeal filed on November 3, 2005, in this application. The appealed claims are as set forth in the attached Claims Appendix. Provision for the payment of fees required for filing this brief, and any required extension of time for filing the brief, are submitted herewith. This brief is submitted in triplicate in accordance with the provisions of 37 C.F.R. §1.192(a).

REAL PARTY IN INTEREST

The real party in interest in this appeal is the CBR Institute for Biomedical Research, Inc., aka The Center for Blood Research, Inc., the assignee of the rights of the inventors in the above-identified patent application. The CBR Institute for Biomedical Research, Inc., is an affiliate of the Harvard Medical School.

RELATED APPEALS AND INTERFERENCES

There are believed to be no related appeals or interferences that will directly affect, be directly affected by, or have a bearing on the Board's decision in this appeal.

STATUS OF CLAIMS

The status of the claims in this application is as follows. Claims 71, 81, 85, 87-89, 92 and 94-97 are pending and are on appeal. Claims 1-70, 72-80, 82-84, 86, 90, 91 and 93 have been canceled. The limitations in claims 71 and 95 directed to mimetics of PSGL-1 has been withdrawn from consideration. Claims 71 and 95 are independent claims. Claim 71 is directed to methods for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on the arterial walls of a mammal. Claim 95 is directed to a method for treating atherosclerosis in a mammal which has been subjected to a vessel corrective technique.

STATUS OF AMENDMENTS

Claims 71, 81, 85, 87-89, 92 and 94-97 were finally rejected in the final Office Action of May 3, 2005. A Notice of Appeal was filed on November 3, 2005. No amendments have been made or entered following issuance of the final Office Action.

SUMMARY OF CLAIMED SUBJECT MATTER

Atherosclerosis is a principal cause of heart attacks among adults in the United States. This condition results from the restricted flow of arterial blood due to the accumulation of fibrous plaque over time in the arterial lumen. Death or incapacity of the subject may result from the rupture of the fibrous cap of the plaque, causing hemorrhage, thrombosis and occlusion of the artery. The fibrous plaque is formed from fatty streaks which develop into lesions composed predominantly of layers of smooth muscle cells, lipid-filled macrophages, and T cells. The earliest stages of atherosclerosis occur when migrating monocytes and T lymphocytes bind to the lumen of the arterial wall. Atherosclerosis is a chronic, long term condition, and is distinguished from more acute conditions such as local inflammation. Page 1, line 17 to page 2, line 22. Appellants have found that P-selectin is implicated in the origins of atherosclerosis as a result of the mediation of platelet or endothelial cell binding and adhesion to monocytes.

In one embodiment of the invention, as described in claim 71, the invention is directed to a method for treating or inhibiting atherosclerosis in a mammal by decreasing the formation or growth of plaque on arterial walls in a mammal. This is accomplished by administering to the mammal an agent selected from the group consisting of PSGL-1, soluble forms of PSGL-1, and fragments of PSGL-1. The agent is administered to the mammal in repeated sequential doses or by controlled release methods over a period of months or years, and is effective to inhibit the interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin. Page 3, lines 1-16; page 4, lines 23-28; page 5, lines 6-14 and 27-32; page 7, lines 1-3; and page 12, line 21 to page 14, line 2.

In another embodiment, as described in claim 95, the invention is directed to a method for treating atherosclerosis in a mammal by performing a vessel corrective technique on a mammal selected form the group consisting of angioplasty, stenting, atherectomy and bypass surgery on the mammal, and subsequently administering to the mammal an effective amount of an agent selected form the group consisting of PSGL-1, soluble forms of PSGL-1, and PSGL-1 fragments. The administration of the agent is accomplished over a period of months or years, and results in a decrease in the formation or growth of plaque on the arterial walls. Page 13, line 21 to page 14, line 2; and page 14, lines 20-29.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether claims 71, 81, 85, 87-89, 92 and 94-97 are unpatentable under 35 U.S.C. 103(a) as obvious over Cummings et al. (U.S. Patent No. 5,464,778) in view of Larsen et al. (U.S. Patent No. 5,840,679), Tedder et al. (U.S. Patent No. 5,834,425), Coller et al. (U.S. Patent No. 5,976,532), Sluiter et al. (*J. Cardiovascular Pharmacology*, 22 (Suppl. 4): S37-S44 (1993)), Aberg et al. (U.S. patent No. 5,061,694), Casscells et al. (U.S. Patent No. 5,308,622), Hinstridge et al. (*Drugs*, 42 (Suppl. 2): 8-2 (1991)), *The Merck Manual of Diagnosis and Therapy*, 16th Ed., pages 409-413 (1992), and De Felice et al. (*Angiology* 41:1-11 (1990))

2. Whether claims 71, 81, 85, 87-89, 92 and 94-97 are unpatentable based on the judicially created doctrine of obviousness-type double patenting in view of claims 40-41, 49-52, 59-60 and 73 of U.S. Patent Application No. 09/436,076.

ARGUMENTS

I. Rejection of Claims 71, 81, 85, 87-89, 92 and 94-97 as obvious over Cummings et al. in view of Larsen et al., Tedder et al., Coller et al., Sluiter et al., Aberg et al., Casscells et al., Hinstridge et al., *The Merck Manual of Diagnosis and Therapy*, and De Felice et al.

The primary reference cited by the Examiner, Cummings et al., discusses atherosclerosis in a section labeled "Clinical Applications". See col. 18, line 33 of the patent. In particular, the Cummings, et al. patent states that atherosclerosis is an example of a pathological condition in which an inflammatory response may occur, and that the P-selectin glycoprotein ligand can be used to treat such an inflammatory responses. See col. 18, lines 34-53 of the reference.

It is appellants' position that Cummings et al. is directed to the treatment of acute inflammatory conditions, such as ischemica and reperfusion, rather than atherosclerosis. The focal point of the reference is the prevention of leukocyte adherence to vascular endothelium. With regard to atherosclerosis, the reference makes the following comments, at col. 19, line 64 to col. 20, line 5:

> "Platelet-leukocyte interactions are believed to be important in atherosclerosis. Platelets might have a role in recruitment of monocytes into atherosclerotic plaques; the

accumulation of monocytes is known to be one of the earliest detectable events during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia."

These comments do not teach or suggest that the reference contemplates the use of PSGL-1 for reducing the formation of arterial plaque. Rather, the reference is directing one skilled in the art to the treatment of thrombus (blood clot) formation. Such treatment would involve the prevention of platelet activation by leukocytes as described elsewhere in the reference. A reduction is plaque formation is not inherent in the treatment of a thrombosis since plaque reduction would require a treatment regime of months or years.

Furthermore, Cummings et al. is directed to the prevention of platelet activation in the circulatory system, rather that the inhibition of endothelial cell binding which is an essential component of atherosclerosis. See, in particular, the Wagner (II)132 Declaration, at paragraphs 4, 5 and 6. Thus, it is appellants' position that one skilled in the art, reading the Cummings et al. reference, would have no reasonable expectation that PSGL-1 could be used to reduce plaque formation, and further, that a long term treatment regime would be required to achieve this result.

Appellants also respectfully submit that the Cummings, et al. reference has been antedated as a result of the prior conception and subsequent reduction to practice of the claimed invention, coupled with the requisite diligence, as shown in the Wagner 131 Declaration.

Larsen et al., like Cummings et al., does not relate to the treatment of chronic conditions, such as atherosclerosis, but is instead directed to the treatment of inflammatory or acute conditions. Contrary to the position taken in the Office Action, neither Cummings et al. nor Larsen et al. disclose that a treatment for atherosclerosis can be administered in conjunction with a vessel-corrective technique.

Coller et al. relates to the treatment of a thrombotic condition using antibodies to GPIIb/IIIa. The present invention, in contrast, relates to the use of PSGL-1, and variants therefore, rather than antibodies. Cummings et al. does not teach the use of vessel corrective techniques, and does not teach the use of antibodies for therapeutic purposes. Consequently, applicants maintain that there is no basis for combining the Coller et al. and Cummings et al. references.

The Sluiter et al. reference has been cited to provide further evidence that one skilled in the art would have targeted the inhibition of P-selectin-mediated events for inhibiting leukocyte adhesion receptors to alleviate tissue damage in cardiovascular diseases. However, although the Sluiter et al. reference mentions P-selectin in a general sense, there is no disclosure in the reference concerning the inhibition of P-selectin binding to the ligand of P-selectin. In fact, the Sluiter et al. reference is actually directed to the possible role of oxygen-derived free radicals in the treatment of inflammation. See the Summary portion of the reference on page S37, and the discussion on page S38. Accordingly, the Sluiter et al. reference does not oversome the shortcomings of Cummings et al.

The remaining references cited by the Examiner do not cure the deficiencies of the Cummings et al. Larsen et al., Coller et al. and Sluiter et al. references as discussed above. references. In particular, the Aberg et al., Casscells et al. and Hinstridge et al. references do not relate to the use of appellant's agent for the treatment of diseases. Accordingly, it would be entirely speculative to suggest that the use of appellants' particular agents for the treatment of atherosclerosis can be administered over a prolonged period of time, and that such treatment would have beneficial results.

Similarly, the Merck and De Felice et al. references are apparently relied upon for linking atherosclerosis with a decrease in plaque growth or formation. Of course, appellants do not claim to have discovered the scientific basis for atherosclerosis. Rather, appellants have developed a treatment protocol for atherosclerosis which is not disclosed or suggested in any of the cited references.

Finally, appellants note that the sheer number of references required for formulating the present obviousness rejection (a total of 10 references) is itself a strong indication that the present claims are not obvious.

II. Rejection of Claims 71, 81, 85, 87-89, 92 and 94-97 as unpatentable based on obviousness-type double patenting in view of claims 40-41, 49-52, 59-60 and 73 of U.S. Patent Application No. 09/436,076).

U.S. Patent Application No. 09/436,076, which forms the basis of this rejection, has now been abandoned. Accordingly, this rejection is now moot.

Summarizing, for the reasons presented in this brief, appellants respectfully urge the Board to reverse the rejection made in the Final Office Action, and to allow all of the appended claims.

Appellants hereby authorize the Commissioner, to debit the $500.00 fee for filing this appeal brief from Appellant's Deposit Account No. 18-1945. If there are any other fees not accounted for above, Appellants hereby authorize the Commissioner to charge the fee to Deposit Account 18-1945.

Respectfully submitted,
GOSZ AND PARTNERS

Date: 01/03/06

William Gosz

William G. Gosz
Reg. No. 27,787
Attorney for Appellants
450 Bedford Street
Lexington, MA 02420

CLAIMS APPENDIX

71. A method for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on arterial walls in a mammal comprising:

providing an agent for inhibiting an interaction between P-selectin and a ligand of P-selectin, said P-selectin being on an endothelial cell; and

administering said agent to a mammal in need of such treatment so as to cause such inhibition to occur, said agent being administered repeatedly in sequential doses or by the controlled release to the mammal over a period of months or years,

wherein said agent is selected from the group consisting of PSGL-1, soluble forms of PSGL-1, fragments of PSGL-1, and mimetics of PSGL-1 which resemble PSGL-1 in shape and charge distribution, said agent being effective to inhibit the interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin.

81. The method of claim 71 wherein said P-selectin can bind to said PSGL-1 in the absence of said agent.

85. The method of claim 71 wherein said agent is administered in combination with other therapeutic agents.

87. The method of claim 71 wherein said mammal is human.

88. The method of claim 71 wherein said agent is administered in a dose of from about 0.01 mg/kg to about 200mg/kg of body weight.

89. The method of claim 71 wherein said agent is administered at a dose of about 100 mg/kg of body weight.

92. The method of claim 95, wherein said vessel-corrective technique is selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery.

94. The method of claim 95, wherein said agent is administered in combination with other therapeutic agents.

95. A method for treating atherosclerosis in a mammal to which a vessel-corrective technique is administered comprising:

performing a vessel-corrective technique selected form the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery on a mammal; and

administering to said mammal, after said vessel-corrective technique, an effective amount of an agent selected from the group consisting of PSGL-1, soluble forms of PSGL-1, fragments of PSGL-1, and mimetics of PSGL-1 which resemble PSGL-1 in shape and charge distribution, said agent being administered repeatedly in sequential doses or by the controlled release to the mammal over a period of months or years to decrease the formation or growth of plaque on the arterial walls of the mammal.

96. The method of claim 71 wherein the agent is administered over a period of years.

97. The method of claim 95 wherein the agent is administered over a period of years.

EVIDENCE APPENDIX

1. Declaration of Denisa Wagner and Robert Johnson Under Rule 131*

2. Declaration of Denisa Wagner under Rule 132**

* Considered and entered by the Examiner on March 18, 2003.

** Considered and entered by the Examiner on February 25, 2005.

Ex. No. 1

ATTORNEY DOCKET NO. CFBF-P03-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/436,076	Art Unit:	1644
Filing Date:	November 8, 1999		
For:	METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Washington, D.C. 20231 on 3/24/03.

Patricia McKenney

BOX AMENDMENT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231
Dear Sir:

DECLARATION UNDER 37 CFR 1.131

We, Denisa D. Wagner and Robert C. Johnson, declare and state as follows:

1. We are the applicants of the above-identified patent application, and the co-inventors of the subject matter disclosed and claimed therein.

2. We are familiar with the present claims of the above-identified application, which are directed to methods for treating or inhibiting atherosclerosis in a mammal by administering an agent that inhibits an interaction between P-selectin and PSGL-1 and E-selectin and a ligand of E-selectin., e.g. PSGL-1 (P-selectin glycoprotein ligand-1), soluble forms of PSGL-1, fragments of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

3. We conceived the claimed invention at least as early as 1988, and coupled with due diligence from a time prior to November 16, 1992, reduced the claimed in invention to practice at least as early as May 6, 1994.

4. Exhibit A is a copy of a page showing a note authored by co-inventor Denisa Wagner in 1988. The notes shown in the Exhibit were recorded by Dr. Wagner during the conference of the American Heart Association held in 1988, and were written on the last page of the program booklet next to a listing of meetings to be held in 1989. The note on the bottom right hand side of the page states that

> Macrophages (Mφ) eat bits of activated platelets. ELAM-1 = Padgem. Do monocytes bind to Padgem on platelets. Padgem is an opsomizing agent to get rid of debris of platelets.

The term "Padgem" here refers to P-selectin and the term "ELAM-1" refers to E-selectin (Endothelial Leukocyte Adhesion Molecule). In 1988, E-selectin was known to mediate endothelial binding to leukocytes. We conceived that there is a functional relationship between E-selectin and P-selectin, and that P-selectin mediates the binding of platelets to macrophages (leukocytes implicated in atherosclerosis).By binding to Padgem, the macrophages are "eating" bits of activated platelets, thereby increasing the fat (lipid) content of the macrophages, and promoting their conversion into foam cells (macrophage cells with a "foamy" appearance due to the presence of lipids that act as precursors for atherosclerotic plaque). Exhibit A thus demonstrates that we had identified a role for P-selectin and E-selectin in some of the key pathological events involved in atherosclerosis, e.g. macrophage binding to P-selectin on platelets, from a time well before November 16, 1992.

5. Exhibit B, also written by Dr. Wagner, describes an experiment we conceived on February 28, 1992. Exhibit B states:

Breed P-selectin deficient mouse with a mouse strain that develops atherosclerosis. See if it (atherosclerosis) can be prevented.

According to this proposed experiment, a mouse deficient in P-selectin would be bred with a mouse strain that develops atherosclerosis to determine whether atherosclerosis can be prevented. In other words, we conceived that if P-selectin/ligand binding and/or E-selectin/ligand binding could be inhibited *in vivo* in a mammal, the atherosclerotic lesions could be reduced or inhibited. In order to complete this experiment, we understood that it would first be necessary to prepare a P-selectin knock-out mouse, and breed this mouse with mouse strains susceptible to atherosclerosis. It is known that mice are generally resistant to developing atherosclerosis. The mouse strain most susceptible to developing atherosclerosis is the C57 black mouse. But the C57 mouse must still be fed a high lipid diet to observe any meaningful development of atherosclerosis.

6. Exhibit C, also written by Dr. Wagner, describes a proposal we conceived on March 2, 1992, to study the role of the P-selectin in atherosclerosis by developing a suitable mouse model, and feeding the P-selectin deficient mice (mutants) and control wild-type mice (P-selectin positive) with a lipid diet. The, formation of atherosclerotic lesions in the mice would be studied and characterized. Exhibit C states, on page 5:

Study the role of P-selectin in atherosclerosis by feeding P-selectin deficient and P-selectin positive mice a lipid diet. Study the formation of atherosclerotic lesions in mice.

Page 5 of Exhibit C also poses the question whether von Willebrand (vW) disease pigs may be resistant to atherosclerosis because of a lack of P-selectin. P-selectin is stored in granules containing vW factor, and these granules are absent in vW disease.

7. At a time prior to November 16, 1992, we undertook to prepare a mouse model for subsequent testing. The mouse model took at least 4 years to prepare, and was completed on or about September 13, 1993. In order to prepare the mouse model, we used a knock-out mouse deficient in P-selectin and back-crossed this mouse with C57 black mice. In order to be sure that the resulting mutant mouse would be susceptible to atherosclerotic lesion development, we

decided to breed 4 generations of mice, with each generation being more susceptible to atherosclerosis. First we developed a P-selectin deficient mouse. Then we bred the P-selectin deficient mouse with a C57 black mouse. Finally, we bred the offspring of the first breeding with another C57 black mouse, and so on for a total of 4 back-cross breedings. We reasoned that the fourth generation would be suitable for evaluation. It took us about 3 years to make a P-selectin deficient mouse, and another year to complete the back-crossing process with the C57 black mice. This work was laborious and continuous, and consumed a large amount of our time and effort. Although the general technology for creating mouse models had been developed by others, we were the first to develop a P-selectin-deficient mouse model. We diligently worked on successfully constructing such a model, and verifying the correct properties and characteristics of the mutant mouse by about September 13, 1993.

8. After the preparation of the mutant mouse deficient in P-selectin on the C57 black background, we promptly commenced feeding the mice (control and experimental) a diet high in lipids. The experimental and control mice were fed a lipid diet for approximately eight months prior to sacrificing the animals and recording the data, This took approximately 8 months since even the C57 black mice are somewhat resistant to the formation of atherosclerosis. Immediately thereafter, we sacrificed the animals and evaluated them for the size and character of atherosclerotic lesions. We prepared the table enclosed as Exhibit D on May 6, 1994. The table in Exhibit D shows the size of atherosclerotic lesions in P-deficient (mutant) mice compared to wild type mice as controls. These results demonstrate a reduction in the size of atherosclerotic lesions in P-selectin deficient mice. Based on these results we concluded that inhibitors of P-selectin/ligand binding and/or E-selectin/ligand binding would be useful for the treatment or inhibition of atherosclerosis, and this constitutes an actual reduction to practice of the claimed invention.

9. From the above information, we deduced that inhibitors of P-selectin and/or E-selectin could be used to treat atherosclerosis in mammals based on the role of P-selectin and/or E-selectin on the pathogenesis of atherosclerosis as presently claimed in the above-identified application. We further believe that the above information constitutes evidence the claimed

invention was conceived prior to November 16, 1992, and diligently reduced to practice at least as early as the actual reduction to practice date of May 6, 1994.

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/25/2003
Date

Denisa D. Wagner

3-6-03
Date

Robert C. Johnson

AMERICAN HEART ASSOCIATION CME OFFERINGS
1989 Highlights

For information contact the American Heart Association, Scientific Sessions, 7320 Greenville Avenue, Dallas, Texas 75231.

***SCIENTIFIC CONFERENCE ON MEMBRANE EVENTS AND INTRACELLULAR SIGNALLING IN THE CARDIOVASCULAR SYSTEM**
Waikoloa, Hawaii
AHA Council on Basic Science and the Japanese Heart Foundation
January 7-11, 1989
Conference Chairman: James T. Stull, PhD

14TH INTERNATIONAL JOINT CONFERENCE ON STROKE AND CEREBRAL CIRCULATION
San Antonio, TX
AHA Council on Stroke
February 9-11, 1989
Conference Chairman: Vladimir C. Hachinski, MD

SCIENTIFIC CONFERENCE ON CORONARY ATHEROSCLEROSIS AND THROMBOSIS
Keystone, CO
AHA Councils on Circulation, Atherosclerosis, Thrombosis, and Clinical Cardiology
February 22-25, 1989
Conference Chairman: Paul J. Cannon, MD

2ND INTERNATIONAL CONFERENCE ON PREVENTIVE CARDIOLOGY AND THE ANNUAL MEETING OF THE AHA COUNCIL ON EPIDEMIOLOGY
Washington, DC
AHA Council on Epidemiology
June 18-22, 1989
Conference Chairman: Jeremiah Stamler, MD

***15TH TEN-DAY SEMINAR ON THE EPIDEMIOLOGY AND PREVENTION OF CARDIOVASCULAR DISEASES**
Tahoe City, CA
AHA Council on Epidemiology
July 30-August 12, 1989
Conference Chairman: Darwin R. Labarthe, MD, PhD

43RD ANNUAL FALL CONFERENCE AND SCIENTIFIC SESSIONS OF THE COUNCIL FOR HIGH BLOOD PRESSURE RESEARCH
Cleveland, OH
AHA Council for High Blood Pressure Research
September 26-29, 1989
Conference Chairman: Allen W. Cowley, Jr, PhD

62ND SCIENTIFIC SESSIONS
New Orleans, LA
AHA Scientific Councils
November 13-16, 1989
Conference Chairman: Michael R. Rosen, MD

*Limited attendance

do plt release by β blocks w $^{125}\beta$ inhibit B/β 15-42

put flow through of ϕ column to on to fibrin column. ϕ rec. should be reduced. control do it reverse go back to fibrin clots does after reacting w fibrin the receptor get phosphorylated

Put on column EC grown in *Phosphate I stimulation w fibrin, EDTA elute If this works to A23 release + incubation etc.
Do x-linking - it will work

Add ϕ to cell lysate put on fibrin column. ϕ should inhibit $IIb\,IIIa$-like binding but fibrin specific b. should not be affected !!

elute w RGD, b. to fibrin may not be through RGD or elute w γ pept.

see if severe cWd pts plts have Padgem

M ϕ eat bits of activated plts ELAM-1 ! = Padgem
do monocytes b. to Padgem on plts
Padgem is an opsonizing agent to get rid of Depres Pt plts

A23187 makes these vesicles are these some of Padgem ?

Role of vicinal cysteines in integrins matrix assembly?

Targeting of P-selectin in yeast

~~Is there a storage compartment in yeast~~

use yeast secretion mutants and clathrin ⊖ cells to find the cellular machinery responsible for targeting of transmembrane proteins.

5/6/94 Denisa Bob

	Genotype	Score
271	Mut	-
278	WT	++ 2.7
279	Mut	-
137	Mut	-
119	WT	+ 0.5
548 E	Mut	+ 0.2
269	WT	++
270	Mut	+ small
40	WT	+++
34	Mut	-
35	WT	+
19	WT	+ (Maybe 2+)
20	WT	2+
268	WT	+++
42	Mut	-
106	WT	+++
18	Mut	-
89	WT	-

Rachelle A. Rosenbaum 5/9/94
City of Boston
Suffolk County
a true copy of the original
MY COMMISSION EXPIRES JUNE 6, 1997

** Sue when it is deep lesion + not raised it is not a lesion. #34

#19 Must be positive about lesion.
Not good

EXHIBIT D
Page 1 of 1

Ex. No. 2

Attorney Docket No. CFBF-P02-002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant(s):	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	November 8, 1999		
For:	METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS WITH PSGL-1		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, P.O. 1450, Alexandria, VA. 22313-1450 on February 22, 2005.

Patricia McKenney

Commissioner for Patents
P.O. 1450
Alexandria, VA. 22313-1450

DECLARATION UNDER RULE 132

Sir:

I, Denisa D. Wagner, declare and state as follows:

1. I am a Professor in the Department of Pathology at Harvard Medical School, and a Senior Investigator at The CBR Institute for Biomedical Research, Inc., Boston, Massachusetts. My Curriculum Vitae is attached hereto as an Exhibit. I am also an inventor of the above-identified patent application. I consider myself to be an expert in the field of cardiovascular medicine and pathology, as reflected in my Curriculum Vitae, and I am well aware of the knowledge level of others skilled in this art.

2. I have reviewed the outstanding Office Action of October 21, 2004, in the above-identified patent application. I am also familiar with the claims of this application, as presently amended, which are directed to methods for treating or inhibiting atherosclerosis by decreasing the formation or growth of plaque on arterial walls. This is accomplished by administering PSGL-1, or selected variants thereof, to a subject over a prolonged period of time, i.e. months or years.

3. I am familiar with the references cited by the Examiner in the outstanding Office Action. In particular, I have reviewed the Cummings et al. reference (U.S. Patent No. 5,464,778), which I understand to be the primary reference cited in the Office Action.

4. The Cummings et al. reference is generally directed to inflammatory thrombotic conditions such as ischemia and reperfusion. In col. 19, line 64 to col. 20, line 5, the Cummings et al. reference makes the following disclosure relating to atherosclerosis and platelet-leukocyte interactions:

> "Platelet-leukocyte interactions are believed to be important in atherosclerosis. Platelets might have a role in recruitment of monocytes into atherosclerotic plaques; the accumulation of monocytes is known to be one of the earliest detectable events during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia."

5. My interpretation of the above cited passage is as follows. Cummings et al. speculate that platelet-leukocyte interactions are important in atherosclerosis. In fact, it is now well established that the key in atherosclerotic lesion development is the direct binding of monocytes to endothelial cells, and the reference does not discuss this. Cummings et al. discusses events following plaque rupture. Such events include thrombus formation leading to ischemic injury causing neutrophil recruitment. This event occurs long after plaque formation which is subject of the present application. The claims of our application specify that the P-selectin is on endothelial cells. Endothelial cells coat the arterial wall, and are not part of the

circulatory system as are the platelets. The plaque rupture, thrombotic events and neutrophil recruitment to the ischemic area discussed in the reference are not part of the present application.

6. I believe that the present invention can be distinguished from the Cummings et al. reference in the following respects. The present invention is directed to the treatment of atherosclerosis by decreasing the formation or growth of plaque on arterial walls. Atherosclerosis is a chronic condition caused by many factors, primarily by excessive plasma cholesterol levels, and results in the deposition of lesions and plaque on arterial walls. The treatment of atherosclerosis requires the long term administration of a medication to a subject in order to result in a meaningful improvement of the condition. This contrast with the treatment of a thrombosis, as disclosed in the Cummings et al. reference, which requires the commencement of an immediate treatment regime in order to prevent the reoccurrence of a thrombotic attack.

7. I also believe that the ability to design a mimetic of PSGL-1 having similar inhibitory characteristics, i.e. the ability to inhibit P-selectin, would be within the skill of a person in the art. Such a mimetic would optimally be designed based on a similarity of charge and shape as stated in the present claims.

8. Based on my knowledge, training and experience, it is my opinion that the references cited in the outstanding Office Action would not teach or suggest the method for treating atherosclerosis as stated in the present claims of the above-identified patent application.

I further declare that statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 2/18/05

Denisa D. Wagner, Ph.D.

CURRICULUM VITAE

DENISA D. WAGNER, Ph.D.

ADDRESS: The CBR Institute for Biomedical Research
Harvard Medical School
800 Huntington Avenue
Boston, MA 02115
Phone: (617) 278-3344
FAX: (617) 278-3368

PLACE OF BIRTH: Prague, Czechoslovakia; U.S. citizen

EDUCATION: Universite de Geneve, Switzerland - Biochemistry
Diploma of Biochemistry, 1975, with distinction

Massachusetts Institute of Technology, Cambridge, MA
Biology - Ph.D., 1980

Harvard University, Cambridge, MA
M.A. (honorary), 1997

FACULTY POSITIONS:

Professor of Pathology, Harvard Medical School, Boston, MA.
1997-present.

Senior Investigator, The CBR Institute for Biomedical Research (formerly known as The Center for Blood Research), Boston, MA.
1994-present.

Associate Professor of Pathology, Harvard Medical School, Boston, MA.
1994-1997.

Associate Professor of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA. 1989-1994.

Associate Professor of Medicine, Tufts University School of Medicine and Member, Special and Scientific Staff, New England Medical Center, Boston, MA. 1987-1994.

Assistant Professor of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1985-1987.

Assistant Professor of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1982-1987.

Senior Instructor in Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. 1981-1982.

AWARDS:

Established Investigator Award, American Heart Association, Biosynthesis of von Willebrand protein by endothelial cells. 1986-1991.

XIth ISTH Congress award in recognition of an outstanding communication, 1987.

Gwendolyn J. Stewart Memorial Award to honor women in the biomedical sciences, 1998.

Special recognition award from the Council on Arteriosclerosis, Thrombosis and Vascular Biology, AHA, 1998

MERIT award, National Heart, Lung and Blood Institute, NIH, 1998-2008.

Sol Sherry Lecture, American Heart Association, 2004.

MAJOR COMMITTEE ASSIGNMENTS:

University:

1991-1994	Member of the Graduate Advisory Committee of the Graduate Program in Cell, Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University
1992-1994	Sackler School Committee on Programs and Faculty, Tufts University
1992-1994	Graduate Admission Committee of the Graduate Program in Cell, Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University
1995-Present	Member of the Graduate Program in Biological and Biomedical Sciences, Harvard Medical School
1998-Present	Member, Committee for Immunology, Program in Immunology, Harvard Medical School
1999-2002	Member of the Faculty Fellowship Committee, Harvard Medical School
2001-2004	Member, Standing Committee on Promotions, Reappointments, and Appointments, Harvard Medical School
2003-Present	Elected Member, Harvard Medical School Faculty Council

National and Regional:

Served on many review committees and panels for the National Institutes of Heath, American Heart Association, Juvenile Diabetes Foundation and American Red Cross.

Currently permanent member, NIH, NHLBI Thrombosis and Hemostasis Study Section (2002-2006)

MEMBERSHIPS, OFFICES, AND COMMITTEE ASSIGNMENTS IN PROFESSIONAL SOCIETIES:

1980-Present	American Society for Cell Biology
1982-Present	American Society of Hematology
1982-Present	International Society of Thrombosis and Haemostasis
1983-1997	Council on Thrombosis, American Heart Association
1985-Present	International Society of Thrombosis and Haemostasis, subcommittee on von Willebrand factor
1991-1996	American Heart Association, Vascular Wall Biology Study Committee

1992-Present	American Heart Association, Council on Thrombosis Executive Committee
1993-1995	American Heart Association, Council on Thrombosis Long-Range Planning Committee
1994-1996	American Heart Association, Council on Thrombosis Membership Committee (Chairman)
1994-Present	American Association for the Advancement of Science
1994-Present	North American Vascular Biology Organization (Founding Member)
1995-1998	American Society of Hematology, Scientific Subcommittee on Thrombosis & Vascular Biology
1997-Present	North American Vascular Biology Organization (Councilor)
1997-Present	Council on Arteriosclerosis, Thrombosis and Vascular Biology, American Heart Association (Fellow)
1998	Council of the Gordon Research Conferences (Member)
1998-Present	The Molecular Medicine Society (Member)
1999-Present	Boston Obesity Nutrition Research Center (Member)
2001-Present	National Hemophilia Foundation (Member)
2001-2005	American Society of Hematology, Scientific Committee on Thrombosis & Vascular Biology (Member)
2002-Present	Harvard Center for Neurodegeneration & Repair (Member)
2004-2010	Council of the International Society on Thrombosis and Haemostasis (Member)
2004-2005	Chair, Scientific Committee on Thrombosis and Vascular Biology, American Society of Hematology

EDITORIAL BOARDS:

1993-2004	Molecular Biology of the Cell
1994-1999	Journal of Clinical Investigation
1998-Present	Molecular Medicine
2003-2008	Blood

PUBLICATIONS
DENISA D. WAGNER, Ph.D.

1. Hynes RO, Ali IU, Destree AT, Mautner V, Perkins ME, Senger DR, **Wagner DD** and Smith KK. A large glycoprotein lost from the surfaces of transformed cells. Ann NY Acad Sci 312:317-342, 1978.

2. **Wagner DD** and Hynes RO. Domain structure of fibronectin and its relation to function (disulfides and sulfhydryl groups). J Biol Chem 254:6746-6754, 1979.

3. Hynes RO, Destree AT, Perkins ME and **Wagner DD.** Cell surface fibronectin and oncogenic transformation. J Supramolecular Str 11:95-104, 1979.

4. **Wagner DD** and Hynes RO. Topological arrangement of the major structural features of fibronectin. J Biol Chem 255:4304-4312, 1980.

5. **Wagner DD,** Ivatt R, Destree AT and Hynes RO. Similarities and differences between fibronectins of normal and transformed hamster cells. J Biol Chem 256:11708-11715, 1981.

6. **Wagner DD** and Hynes RO. Fibronectin coated beads are endocytosed by cells and align with microfilament bundles. Exp Cell Res 140:373-381, 1982.

7. Van De Water L III, **Wagner DD,** Crenshaw EB III and Hynes RO. Fibronectin-dependent endocytosis by macrophage-like ($P388D_1$) and fibroblastic (NIL 8) cells. In: Cellular Recognition. Glaser L, Frazier W and Gottlieb D (Eds.). New York: Alan R. Liss, 869-878, 1982.

8. Hynes RO, Destree AT and **Wagner DD.** Relationships between microfilaments, cell-substratum adhesion and fibronectin. Cold Spring Harbor Symposia on Quantitative Biology 46:659-669, 1982.

9. **Wagner DD,** Olmsted JB and Marder VJ. Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. J Cell Biol 95:355-360, 1982.

10. **Wagner DD** and Marder VJ. Biosynthesis of von Willebrand protein by human endothelial cells: identification of a large precursor polypeptide chain. J Biol Chem 258:2065-2067, 1983.

11. **Wagner DD,** Urban-Pickering M and Marder VJ. von Willebrand protein binds to extracellular matrices independently of collagen. Proc Natl Acad Sci USA 81:471-475. 1984.

12. Sporn LA, Rubin P, Marder VJ and **Wagner DD.** Irradiation induces release of von Willebrand protein from endothelial cells in culture. Blood 64:567-570, 1984.

13. **Wagner DD** and Marder VJ. Biosynthesis of von Willebrand protein by human endothelial cells: processing steps and their intracellular localization. J Cell Biol 99:2123-2130, 1984.

14. Sporn LA, Chavin SI, Marder VJ and **Wagner DD.** Biosynthesis of von Willebrand protein by human megakaryocytes. J Clin Invest 76:1102-1106, 1985.

15. **Wagner DD,** Mayadas T, Urban-Pickering M, Lewis BH and Marder VJ. Inhibition of disulfide bonding of von Willebrand protein by monensin results in small, functionally defective multimers. J Cell Biol 101:112-120, 1985.

16. Fay PJ, Kawai Y, **Wagner DD,** Ginsberg D, Bonthron D, Ohlsson-Wilhelm BM, Chavin SI, Abraham GN, Handin RI, Orkin SH, Montgomery RR and Marder VJ. Propolypeptide of von Willebrand factor circulates in blood and is identical to von Willebrand antigen II. Science 232:995-998, 1986.

17. Sporn LA, Marder VJ and **Wagner DD.** Inducible secretion of large biologically potent von Willebrand factor multimers. Cell 46:185-190, 1986.

18. **Wagner DD,** Mayadas T and Marder VJ. Initial glycosylation and acidic pH in the Golgi apparatus are required for multimerization of von Willebrand factor. J Cell Biol 102:1320-1324, 1986.

19. **Wagner DD,** Lawrence SO, Ohlsson-Wilhelm BM, Fay PJ and Marder VJ. Topology and order of formation of interchain disulfide bonds in von Willebrand factor. Blood 69:27-32, 1987.

20. **Wagner DD,** Fay PJ, Sporn LA, Sinha S, Lawrence SO and Marder VJ. Divergent fates of von Willebrand factor and its propolypeptide (von Willebrand antigen II) after secretion from endothelial cells. Proc Natl Acad Sci USA 84:1955-1959, 1987.

21. Sporn LA, Marder VJ and **Wagner DD.** von Willebrand factor released from Weibel-Palade bodies binds more avidly to extracellular matrix than that secreted constitutively. Blood 69:1531-1534, 1987.

22. Sinha S and **Wagner DD.** Intact microtubules are necessary for complete processing, storage and regulated secretion of von Willebrand factor by endothelial cells. Eur J Cell Biol 43:377-383, 1987.

23. Ribes JA, Francis CW and **Wagner DD.** Fibrin induces release of von Willebrand factor from endothelial cells. J Clin Invest 79:117-123, 1987.

24. Mayadas TN, **Wagner DD** and Simpson PJ. von Willebrand factor biosynthesis and partitioning between constitutive and regulated pathways of secretion after thrombin stimulation. Blood 73:706-711, 1989.

25. Handin RI and **Wagner DD.** von Willebrand factor: molecular and cellular biology. In: Progress in Hemostasis and Thrombosis (vol. 9). Coller B (Ed.). Philadelphia: W B Saunders, 233-259, 1989.

26. Roarke MC, **Wagner DD,** Marder VJ and Sporn LA. Temperature-sensitive steps in the secretory pathway for von Willebrand factor in endothelial cells. Eur J Cell Biol 48:337-343, 1989.

27. Sporn LA, Marder VJ and **Wagner DD.** Differing polarity of the constitutive and regulated secretory pathways for von Willebrand factor in endothelial cells. J Cell Biol 108:1283-1289, 1989.

28. Bonfanti R, Furie BC, Furie B and **Wagner DD.** PADGEM (GMP-140) is a component of Weibel-Palade bodies of human endothelial cells. Blood 73:1109-1112, 1989.

29. Avissar N, Whitin JC, Allen PZ, **Wagner DD,** Liegey P and Cohen HJ. Plasma selenium dependent glutathione peroxidase: cell of origin and secretion. J Biol Chem 264:15850-15855, 1989.

30. Ribes JA, Ni F, **Wagner DD** and Francis CW. Mediation of fibrin-induced release of von Willebrand factor from cultured endothelial cells by the fibrin β chain. J Clin Invest 1989, 84:435-442, 1989.

31. Mayadas TN and **Wagner DD.** In vitro multimerization of von Willebrand factor is triggered by low pH: importance of the propolypeptide and free sulfhydryls. J Biol Chem 264:13497-13503, 1989.

32. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, **Wagner DD** and Furie B. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 59:305-312, 1989.

33. **Wagner DD.** Storage and secretion of von Willebrand factor. In: Role of Factor VIII and von Willebrand Factor. Zimmerman TS and Ruggeri ZM. (Eds.). New York: Marcel Dekker, pp. 161-180, 1989.

34. Larsen E, Palabrica T, Sajer S, Gilbert GE, **Wagner DD,** Furie BC and Furie B. PADGEM-dependent adhesion of activated platelets to monocytes and neutrophils is mediated by a lineage-specific carbohydrate, lacto-N-fucopentaose III (CD15). Cell 63:467-474, 1990.

35. **Wagner DD.** Cell biology of von Willebrand factor. In: Annual Review of Cell Biology. Palade GE, Alberts BM and Spudich JS. (Eds). 6:217-246, 1990.

36. Peters JH, Sporn LA, Ginsberg MH and **Wagner DD.** Human endothelial cells synthesize, process, and secrete fibronectin molecules bearing an alternatively spliced type III homology (ED1). Blood 75:1801-1808, 1990.

37. Mayadas TN and **Wagner DD.** von Willebrand factor biosynthesis and processing. Ann NY Acad Sci, 614:153-166, 1991.

38. Furie B, Celi A, Palabrica TM, Larsen E, **Wagner DD** and Furie, BC. PADGEM a Leukocyte Receptor on Activated Platelets: Biology and application to in vivo medical diagnostics. In: Biotechnology of Plasma Proteins: Haemostasis, Thrombosis and Iron Proteins. Albertini A, Lenfant CL, Mannucci PM, and Sixma JJ. (Eds). Basel: Karger, 32-36, 1991.

39. **Wagner DD** and Bonfanti R. von Willebrand factor and the endothelium. Mayo Clinic Proceedings 66:621-627, 1991.

40. Bevilacqua E, Butcher B, Furie B, Furie B, Gallatin M, Gimbrone M, Harlan J, Kishimoto K, Lasky L, McEver R, Paulson J, Rosen S, Seed B, Springer T, Stoolman L, Tedder T, Varki A, **Wagner DD,** Weissman I and Zimmerman G. Letter to the Editor. Selectins: A family of adhesion receptors. Cell 67:233, 1991.

41. **Wagner DD.** Structure and biology of von Willebrand factor. In: Hematology: Basic Principles and Practices. Benz EJ, Cohen HJ, Furie B, Hoffman R and Shattil SJ. (Eds.). New York: Churchill Livingstone, 1354-1358, 1991.

42. **Wagner DD,** Saffaripour S, Bonfanti R, Sadler JE, Cramer EM, Chapman B and Mayadas TN. Induction of specific storage organelles by von Willebrand factor propolypeptide. Cell 64:403-413, 1991.

43. Erban JK and **Wagner DD.** A 130 kDa protein on endothelial cells binds to amino acids 15-42 of the Bβ chain of fibrinogen. J Biol Chem 267:2451-2458, 1992.

44. Mayadas TN and **Wagner DD.** Vicinal cysteines in the prosequence play a role in von Willebrand factor multimer assembly. Proc Natl Acad Sci USA 89:3531-3535, 1992.

45. Larsen GR, Sako D, Ahern TJ, Shaffer M, Erban JK, Sajer SA, Gibson RM, **Wagner DD,** Furie BC and Furie B. P-Selectin and E-Selectin: Distinct but overlapping leukocyte ligand specificities. J Biol Chem 267:11104-11110, 1992.

46. Koedam JA, Cramer EM, Briend E, Furie B, Furie BC and **Wagner DD**. P-selectin, a granule membrane protein of platelets and endothelial cells, follows the regulated secretory pathway in AtT-20 cells. J Cell Biol 116:617-625,1992.

47. Stone JP and **Wagner DD**. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. J Clin Invest 92:804-813, 1993.

48. Vischer UM and **Wagner DD**. CD63 is a component of Weibel-Palade bodies of human endothelial cells. Blood 82:1184-1191, 1993.

49. Subramaniam M, Koedam JA and **Wagner DD**. Divergent fates of P- and E-selectins after their expression on the plasma membrane. Molec Biol of the Cell 4:791-801, 1993.

50. Journet AM, Saffaripour S and **Wagner DD**. Requirement for both D domains of the propolypeptide in von Willebrand factor multimerization and storage. Thromb Haemost 70:1053-1057,1993.

51. Mayadas TN, Johnson RC, Rayburn H, Hynes RO and **Wagner DD**. Leukocyte rolling and extravasation are severely compromised in P-selectin-deficient mice. Cell 74:541-554, 1993.

52. **Wagner DD**. Weibel-Palade body: The storage granule for von Willebrand factor and P-selectin. State of the Art book, Thromb Haemost 70:105-110, 1993.

53. Journet AM, Saffaripour S, Cramer EM, Tenza D, and **Wagner DD**. von Willebrand factor storage requires intact prosequence cleavage site. Eur J Cell Biol 60:31-41, 1993.

54. Vischer UM and **Wagner DD**. von Willebrand factor proteolytic processing and multimerization precede the formation of Weibel-Palade bodies. Blood 83:3536-3544, 1994.

55. **Wagner DD** and Hynes RO. Gene targeting of adhesion molecules in the vasculature. Series: Molecular genetics and gene therapy of cardiovascular diseases. Mockrin SC (Ed.) New York: Marcel Dekker, pp. 403-425, 1995.

56. Stone JP and **Wagner DD**. P-selectin-deficient mice: A model to study inflammation and metastasis. In: Series: Topics in Molecular Medicine Vol 1. Siess W, Lorenz R and Weber PC. (Eds.). New York: Raven Press, pp. 105-116, 1995.

57. **Wagner DD**. P-selectin knock-out: a mouse model for various human diseases. In: Cell adhesion and human disease. Ciba Foundation Symposium 189. Marsh J and Goode J (Eds). Chichester: John Wiley & Sons, pp. 2-16, 1995.

58. **Wagner DD** and Ginsburg D. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 2nd Edition. Benz EJ, Cohen HJ, Furie B, Hoffman R and Shattil SJ. (Eds.). New York: Churchill Livingstone, 112:1717-1725, 1995.

59. Johnson RC, Mayadas TN, Frenette PS, Mebius RE, Subramaniam M, Lacasce A, Hynes RO and **Wagner DD**. Blood cell dynamics in P-selectin-deficient mice. Blood 86:1106-1114, 1995.

60. Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO and **Wagner DD**. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. J Exp Med 181:2277-2282, 1995.

61. **Wagner, DD**. P-selectin chases a butterfly. Editorial. J Clin Invest 95:1955-1956, 1995.

62. Frenette PS, Johnson RC, Hynes RO and **Wagner DD.** Platelets roll on stimulated endothelium *in vivo*: An interaction mediated by endothelial P-selectin. Proc Natl Acad Sci USA 92:7450-7454, 1995.

63. Yamada S, Mayadas TN, Yuan F, **Wagner DD,** Hynes RO, Melder RJ and Jain RK. Rolling in P-selectin deficient mice is reduced but not eliminated in the dorsal skin. Blood 86:3487-3492, 1995.

64. Pinsky DJ, Naka Y, Liao H, Oz MC, **Wagner DD**, Mayadas TN, Johnson RC, Hynes RO, Heath M, Lawson CA and Stern DM. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies: A mechanism for rapid neutrophil recruitment following cardiac preservation. J Clin Invest 97:493-500, 1996.

65. Mayadas TN, Mendrick DL, Brady HR, Tang T, Papayianni A, Assmann KJM, **Wagner DD**, Hynes RO and Cotran RS. Acute passive anti-glomerular basement membrane nephritis in P-selectin-deficient mice: Increased neutrophil influx and proteinuria and decreased lipoxin A_4 generation. Kidney Int 49:1342-1349,1996.

66. Subramaniam M, Frenette PS, Saffaripour S, Johnson RC, Hynes RO and **Wagner DD**. Defects in hemostasis in P-selectin-deficient mice. Blood 87:1238-1242, 1996.

67. Frenette PS, Mayadas TN, Rayburn H, Hynes RO and **Wagner DD**. Susceptibility to infection and altered hematopoiesis in mice deficient in both P-and E-selectins. Cell 84:563-574, 1996.

68. Tang T, Frenette PS, Hynes RO, **Wagner DD** and Mayadas TN. Cytokine-induced meningitis is dramatically attenuated in mice deficient in endothelial selectins. J Clin Invest 97:2485-2490, 1996.

69. Frenette PS and **Wagner DD**. Adhesion Molecules - Part 1. New Engl J Med 334:1526-1529, 1996.

70. Frenette PS and **Wagner DD**. Adhesion Molecules - Part II: Blood vessels and blood cells. New Engl J Med 335:43-45, 1996.

71. Hynes RO and **Wagner DD**. Genetic manipulation of vascular adhesion molecules in mice. J Clin Invest 98:2193-2195, 1996.

72. Johnson RC, Chapman SM, Dong ZM, Ordovas JM, Mayadas TN, Herz J, Hynes RO, Schaefer EJ and **Wagner DD**. Absence of P-selectin reduces fatty streak formation in mice. J Clin Invest 99:1037-1043, 1997.

73. Subramaniam M, Saffaripour S, Van De Water L, Frenette PS, Mayadas TN, Hynes RO and **Wagner DD.** Role of endothelial selectins in wound repair. Am J Path 150:1701-1709, 1997.

74. Frenette PS and **Wagner DD**. Insights into selectin function from knockout mice. Thromb Haemost, State-of-the-Art Issue, 78:60-64, 1997.

75. Dong ZM, Gutierrez-Ramos JC, Coxon A, Mayadas TN and **Wagner DD**. A new class of obesity genes encodes leukocyte adhesion receptors. Proc Natl Acad Sci USA 94:7526-7530, 1997.

76. Walter UM, Ayer LM, Wolitzky BA, **Wagner DD**, Hynes RO, Manning AM and Issekutz AC. Characterization of a novel adhesion function blocking monoclonal antibody to rat/mouse P-selectin generated in the P-selectin-deficient mouse. Hybridoma 16: 249-257, 1997.

77. Walter UM, Ayer LM, Manning AM, Frenette PS, **Wagner DD**, Hynes RO, Wolitzky BA and Issekutz AC. Generation and characterization of a novel adhesion function blocking monoclonal antibody recognizing both rat and mouse E-selectin. Hybridoma 16: 355-361, 1997.

78. Weiser MR, Pechet TTV, Williams JP, Ma M, Frenette PS, Moore FD, Kobzk L, Hynes RO, **Wagner DD**, Carroll MC and Hechtman HB. Experimental murine acid aspiration injury is mediated by neutrophils and the alternative complement pathway. J Appl Physiol 83:1090-1095, 1997.

79. Dong ZM and **Wagner DD**. Leukocytes fighting against obesity. Molecular Psychiatry 3:8-9, 1998.

80. Frenette PS, Moyna C, Hartwell DW, Lowe JB, Hynes RO and **Wagner DD**. Platelet-endothelial interactions in inflamed mesenteric venules. Blood 91:1318-1324, 1998.

81. de Mora F, Williams CMM, Frenette PS, **Wagner DD**, Hynes RO, and Galli SJ. P- and E-selectins are required for the leukocyte recruitment, but not the tissue swelling, associated with IgE- and mast cell-dependent inflammation in mouse skin. Laboratory Investigation 78:497-505, 1998.

82. Fleming JC, Berger G, Guichard J, Cramer EM, and **Wagner DD**. The transmembrane domain enhances granular targeting of P-selectin. Euro J Cell Biol 75:331-343, 1998.

83. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, and **Wagner DD**. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest 102:145-152, 1998.

84. Denis C, Methia N, Frenette PS, Rayburn H, Ullman-Culleré M, Hynes RO, and **Wagner DD**. A mouse model of severe von Willebrand disease: Defects in hemostasis and thrombosis. Proc Natl Acad Sci USA 95:9524-9529, 1998.

85. Seiler KP, Ma Y, Weis JH, Frenette PS, Hynes RO, **Wagner DD**, and Weis JJ. E- and P-selectin are not required for resistance to severe murine Lyme arthritis. Infection and Immunity. 66:4557-2559, 1998.

86. Homeister JW, Zhang M, Frenette PS, Hynes RO, **Wagner DD**, Lowe JB, and Marks RM. Overlapping functions of E- and P-Selectin in neutrophil recruitment during acute inflammation. Blood. 92:2345-2352, 1998.

87. Hartwell D, Butterfield CE, Frenette PS, Kenyon BM, Hynes RO, Folkman J, and **Wagner DD**. Angiogenesis in P-and E-Selectin deficient mice. Microcirculation. 5:173-178, 1998.

88. Dong ZM and **Wagner DD**. Leukocyte-endothelial adhesion molecules in atherosclerosis. J Lab Clin Med 132:369-375, 1998.

89. Mazo IB, Gutierrez-Ramos JC, Frenette PS, Hynes RO, **Wagner DD**, and von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: Parallel contributions of endothelial selectins and VCAM-1. J Exp Med 18:465-474, 1998.

90. Berger G, Hartwell DW and **Wagner DD**. P-selectin and platelet clearance. Blood: 92:4446-4452, 1998.

91. Hartwell DW, Mayadas T, Frenette PS, Berger G, Rayburn H, Hynes RO and **Wagner DD**. Role of P-selectin cytoplasmic domain in granular targeting in vivo and in early inflammatory responses. J Cell Biol 143: 129-1141, 1998.

92. Frenette PS, Subbarao S, Mazo IB, von Andrian UH and **Wagner DD**. Endothelial selectins and VCAM-1 promote hematopoietic progenitor homing to bone marrow. Proc Natl Acad Sci USA 95:14423-14428, 1998.

93. Jia GQ, Gonzalo JA, Hidalgo A, **Wagner D**, Cybulsky M and Gutierrez-Ramos JC. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA4/VCAM-1 interactions. Int Immunol 11:1-10, 1999.

94. Shi C, Feinberg MW, Zhang D, Patel A, Sim CU, Dong ZM, Chapman SC, Gutierrez-Ramos JC, **Wagner DD**, Sibinga NES and Haber E. Donor MHC and Adhesion Molecules in Transplant Arteriosclerosis. J Clin Invest 103:469-474, 1999.

95. Soriano SG, Wang YF, **Wagner DD** and Frenette PS. P- and E-selectin -deficient mice are susceptible to cerebral ischemia-reperfusion injury. Brain Res 835:360-364, 1999.

96. Robinson SD, Frenette PS, Rayburn H, Cummiskey M, Ullman-Culleré M, **Wagner DD**, and Hynes RO, Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. Proc Natl Acad Sci USA 96:11452-11457, 1999.

97. Enjyoji K, Sévigny J, Lin Y, Frenette PS, Christie PD, Schulte am Esch J, Imai M, Edelberg JM, Rayburn H, Lech M, Beeler DL, Csizmadia E, **Wagner DD**, Robson SC and Rosenberg RD. Targeted disruption of *cd39*/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nature Med 5: 1010-1017, 1999.

98. Hartwell, DW and **Wagner DD**. New discoveries with mice mutant in endothelial and platelet selectins. Thromb Haemost, State-of-the-Art Issue, 82:850-857, 1999.

99. Denis C and **Wagner DD**. Insights from von Willebrand disease animal models. Cell Mol Life Sci 56: 977-990, 1999.

100. Methia N, Denis C, and **Wagner DD**. Carboxypeptidase E does not mediate von Willebrand factor targeting to storage granules. Euro J Cell Biol 78:884-891, 1999.

101. Ginsburg D and **Wagner DD**. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 3rd Edition. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE and McGlave P. (Eds.). New York: Churchill Livingstone, 1999.

102. Dong ZM, Brown AA and **Wagner DD**. The prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. Circulation 101:2290-2295, 2000.

103. Frenette PS, Denis CV, Weiss L, Subbarao S, Hartwig JH, Vestweber D and **Wagner DD**. P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. J Exp Med 191:1413-1422, 2000.

104. Ni H, Denis CV, Subbarao S, Degen JL, Sato TN, Hynes RO and **Wagner DD**. Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. J Clin Invest 106:385-392, 2000.

105. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM and **Wagner DD**. Platelets adhere and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood 96:3322-3328, 2000.

106. Andre P, Hartwell D, Hrachovinova I, Saffaripour S and **Wagner DD**. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. Proc Natl Acad Sci USA 97:13835-13840, 2000.

107. Denis CV, Kwack K, Saffaripour S, Maganti S, Andre P, Schaub RG and **Wagner DD**. Interleukin-11 significantly increases plasma von Willebrand factor and factor VIII in wild-type and von Willebrand disease mouse models. Blood 97:465-472, 2001.

108. Denis CV, André P, Saffaripour S and **Wagner DD**. Defect in regulated secretion of P-selectin leukocyte recruitment in von Willebrand factor-deficient mice. Proc Natl Acad Sci USA, 98:4072-4077, 2001.

109. Niederman R, Westernoff T, Lee C, Mark LL, Kawashima N, Ullman-Culler M, Dewhirst FE, Paster BJ, **Wagner DD**, Mayadas T, Hynes RO and Stashenko P. Infection-mediated early-onset periodontal disease in P/E-selectin-deficient mice. J Clin Periodontal 28:569-575, 2001.

110. Ni H, Ramakrishnan V, Ruggeri ZM, Papalia JM, Phillips DR and **Wagner DD**. Increased thrombogenesis and embolus formation in mice lacking glycoprotein V. Blood 98:368-373, 2001.

111. Methia N, André P, Denis CV, Economopoulos M and **Wagner DD**. Localized reduction of atherosclerosis in von Willebrand factor-deficient mice. Blood 98:1424-1428, 2001.

112. Methia N, André P, Hafezi-Moghadam A, Economopoulos M, Thomas KL and **Wagner DD**. ApoE deficiency compromises the blood brain barrier especially after injury. Mol Med 7:810-815, 2001.

113. Gotte M, Joussen AM, Klein C, Andre P, **Wagner DD**, Hinkes MT, Kirchhof B, Adamis AP, and Bernfield M. Role of syndecan-1 in leukocyte-endothelial interactions in the ocular vasculature. Invest Ophthalmol Vis Sci 43:1135-1141, 2002.

114. André P, Prasad KSS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR and **Wagner DD**. CD40L stabilizes arterial thrombi by a β3 integrin-dependent mechanism. Nature Med. 8:247-252, 2002.

115. Sethi S, Ziouzenkova O, Ni H, **Wagner DD**, Plutzky J and Mayadas TN. Oxidized omega-3 fatty acids in fish oil inhibit leukocyte-endothelial interactions through activation of PPARα. Blood 100:1340-1346, 2002.

116. Hoffmeister KM, Felbinger TW, Falet H, Denis CV, Bergmeier W, Mayadas TN, von Andrian UH, **Wagner DD**, Stossel TP and Hartwig JH. The clearance mechanism of chilled blood platelets. Cell 112: 87-97, 2003.

117. Ni H, Yuen PST, Papalia JM, Trevithick JE, Sakai T, Fässler R, Hynes RO and **Wagner DD**. Plasma fibonectin promotes thrombus growth and stability in injured arterioles. Proc Natl Acad Sci USA 100: 2415-2419, 2003.

118. Burger PC and **Wagner DD**. Platelet P-selectin facilitates atherosclerotic lesion development. Blood 101:2661-2666, 2003.

119. Italiano JE Jr, Bergmeier W, Tiwari S, Falet H, Hartwig JH, Hoffmeister K, André P, **Wagner DD** and Shivdasani RA. Mechanisms and implications of platelet discoid shape. Blood 101:4789-96, 2003.

120. Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian HH Jr, Schaub RG, McEver RP and **Wagner DD**. P-selectin/PSGL-1 interaction generates microparticles that correct hemostasis in a murine model of hemophilia A. Nat Med 9:1020-1025, 2003.

121. Matsushita K, Morrell CN, Cambien B, Yang SX, Yamakuchi M, Bao C, Hara M, Quick RA, Cao W, O'Rourke B, Lowenstein JM, Pevsner J, **Wagner DD** and Lowenstein CJ. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. Cell 115:1-20, 2003.

122. **Wagner DD.** Shear madness in TNF-α signaling. Blood 102:2711-2712, 2003.

123. Myers DD, Hawley AE, Farris DM, Wrobelski SK, Thanaporn P, Schaub RG, **Wagner DD**, Kumar A and Wakefield TW. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg 38: 1075-1089, 2003.

124. Ni H, Papalia JM, Degen JL and **Wagner DD**. Control of thrombus embolization and fibronectin internalization by integrin αIIbβ3 engagement of the fibrinogen γ chain. Blood 102: 3609-3614, 2003.

125. Bergmeier W, Burger PC, Piffath CL, Hoffmeister KM, Hartwig JH, Nieswandt B and **Wagner DD**. Metalloproteinase inhibitors improve the recovery and hemostatic function of in vitro-aged or -injured mouse platelets. Blood 102: 4229-4235, 2003.

126. Cambien B, Bergmeier W, Saffaripour S, Mitchell HA and **Wagner DD**. Anti-thrombotic activity of TNF-α. J Clin Invest 112: 1589-1596, 2003.

127. **Wagner DD** and Burger PC. Platelets in inflammation and thrombosis. Arterioscler Thromb Vasc Biol 23: 2131-2137, 2003.

128. Cambien B and **Wagner DD**. A new role in hemostasis for the adhesion receptor P-selectin. Trends Mol Med 10: 179-186, 2004.

129. Bergmeier W, Rabie T, Strehl A, Piffath CL, Prostredna M, **Wagner DD** and Nieswandt B. GPVI down-regulation in murine platelets through metalloproteinase-dependent shedding. Thromb Haemost 91: 951-958. 2004.

130. Kondo T, Hafezi-Moghadam A, Thomas K, **Wagner DD** and Kahn CR. Mice lacking insulin or insulin-like growth factor 1 receptors in vascular endothelial cells maintain normal blood-brain barrier. Biochem Biophys Res Commun 317: 315-320, 2004.

131. Crittenden JR, Bergmeier W, Zhang Y, Piffath CL, Liang Y, **Wagner DD**, Housman DE and Graybiel AM. CalDAG-GEFI integrates signaling for platelet aggregation and thrombus formation. Nat Med 10: 982-986, 2004.

132. Bergmeier W, Piffath CL, Cheng G, Dole VS, Zhang Y, von Andrian UH and **Wagner DD**. Tumor necrosis factor-α-converting enzyme (ADAM17) mediates GPIbα shedding from platelets in vitro and in vivo. Circ Res 95: 677-683, 2004.

133. Ginsburg D and **Wagner DD**. Structure, biology and genetics of von Willebrand factor. In: Hematology: Basic Principles and Practices. 4th Edition. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE and McGlave P. (Eds.). New York: Churchill Livingstone, 2004.

134. Babic AM, Wang HW, Lai MJ, Daniels TG, Felbinger TW, Burger PC, Stricker-Krongrad A and **Wagner DD**. ICAM-1 and β2 integrin deficiency impairs fat oxidation and insulin metabolism during fasting. In press, Mol Med.

135. Wang, HW, Babic, AM, Mitchell HA, Liu K and **Wagner DD**. Elevated soluble ICAM-1 levels induce immune deficiency and increase adiposity in mice. In press, FASEB J.

RELATED PROCEEDINGS APPENDIX

None